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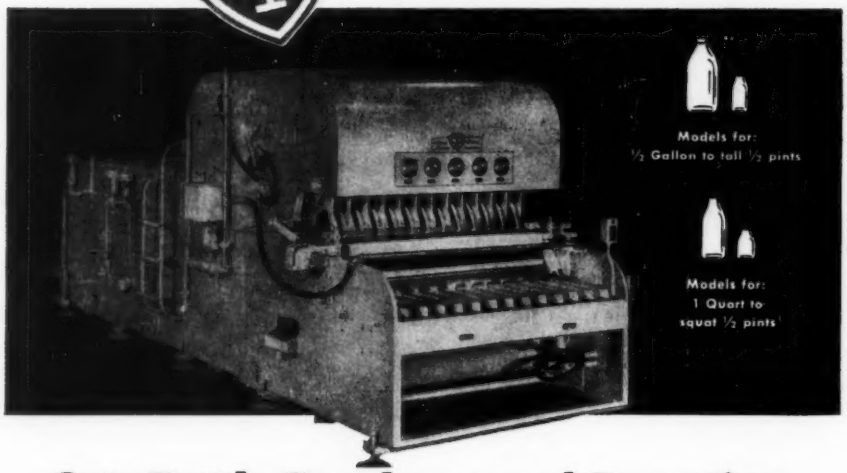

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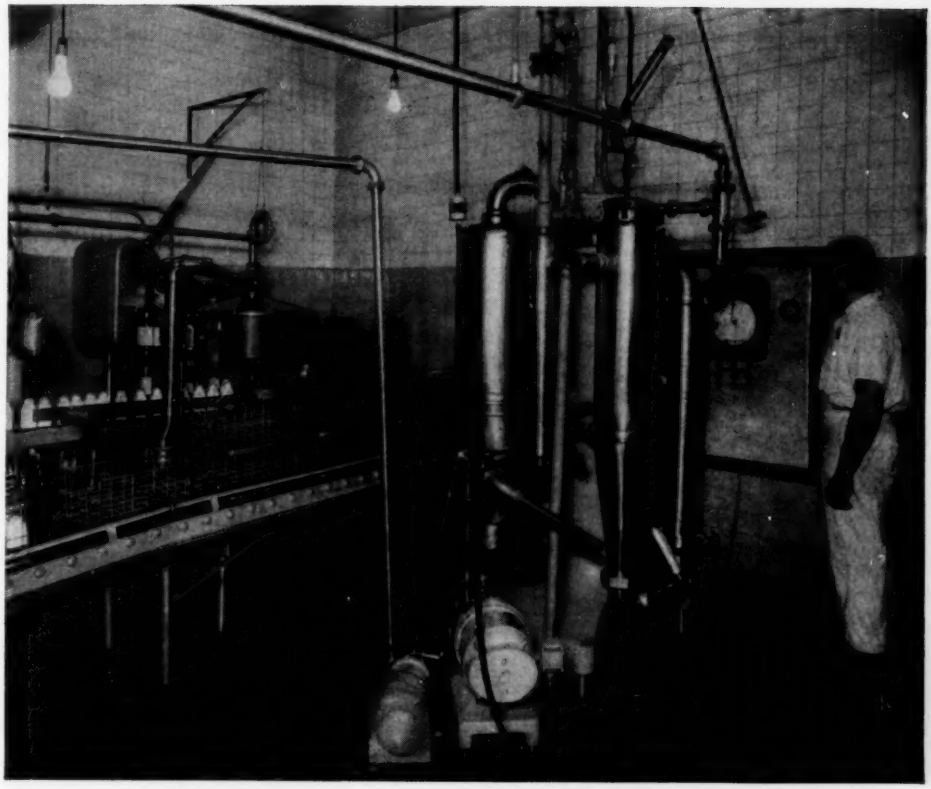

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXVIII APRIL, 1955 NUMBER 4

TOCOPHEROL CONTENT OF CANADIAN BUTTERFAT AND ITS USE IN DETECTING ADULTERATION

CONSTANCE ANGLIN, J. H. MAHON, AND R. A. CHAPMAN

Department of National Health and Welfare, Food and Drug Laboratories, Ottawa, Ontario

A rapid procedure for determining the tocopherol content of butterfat has been developed *(5)*. This method has proved useful in detecting the adulteration of butter, because the tocopherol content of butterfat is low, whereas that of most vegetable fats and oils, with the exception of coconut oil, is considerably higher. Usually, therefore, the adulteration of butter with hydrogenated vegetable fats will result in a significant increase in the tocopherol content of the adulterated fat.

The literature indicates that although the tocopherol content of butterfat is low, it is, nevertheless, subject to considerable seasonal variation. Harris *et al. (4)* found the mean tocopherol content to be 42 p.p.m. for summer milk fat and 23 p.p.m. for winter milk fat. Parrish *et al. (6)* reported a tocopherol content as low as 8 for milk fat. In a more recent survey, Bird *et al. (1)* found the tocopherol contents for milk fat to range from 10 to 45 p.p.m. The foregoing data indicate tocopherol contents of 8 to 45 p.p.m. for butterfat with seasonal variation accounting for a large portion of this range. Since these data do not apply to Canadian butterfat, it was considered desirable to initiate a survey to establish the range and seasonal variation of the tocopherol content of Canadian commercial butterfat by employing our tocopherol procedure *(5)*.

EXPERIMENTAL PROCEDURE AND RESULTS

Butter samples were collected at monthly intervals from 29 selected manufacturers located in eight provinces over a period of 1 year. Each figure reported in Table 1 represents the average of two analyses conducted on the same butterfat sample. The butter from which these samples were taken was churned under the supervision of Provincial dairy instructors. It should be emphasized that the factories supplying these samples derived their milk from large numbers of cows (28,000 to 56,000) which were fed, housed, and milked according to commercial practices. It is believed, therefore, that the results of this survey are representative of Canadian commercial butterfat production for the period June, 1953, to June, 1954. These results are given in Table 1.

Received for publication September 7, 1954.

TABLE 1

Tocopherol content (p.p.m.) of genuine Canadian butterfats for the period June, 1953, to June, 1954

Source of butterfat		Month of butter manufacture												
		1953							1954					Av. by towns[a]
Province	Town	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	
		(Parts per million)												
Nova Scotia	Middle Musquodoboit	34	45	50	41	40	27	29	20	18	17	20	23	30
	Antigonish	35	45	45	42	46	39	29	19	—	21	21	25	32
	Stellarton	35	46	49	40	42	36	32	16	19	20	18	29	32
	Tatamagouche	36	46	48	44	45	29	29	19	17	17	—	19	31
New Brunswick	Sussex	32	—	42	42	42	36	33	18	22	21	25	23	31
	St. Hilaire	29	38	43	40	35	—	—	19	22	—	23	—	29
	Moncton	28	38	43	40	40	38	32	18	19	20	17	—	30
Quebec	Kirouac	37	41	43	42	41	28	—	17	16	20	19	19	29
	Louiseville	40	43	46	45	44	34	—	22	17	21	24	28	33
	St. Urbain de Chateauguay	40	42	42	45	37	25	—	—	13	18	22	27	29
	St. Victoire	35	39	41	38	41	29	—	—	—	—	—	—	—
Ontario	Fenelon Falls	38	40	43	46	40	36	35	24	20	20	18	38	33
	Sutton West	31	40	44	44	45	29	28	22	17	15	17	35	31
	Jarvis	38	44	45	44	44	25	28	21	20	18	15	20	30
	Earlton	34	40	45	39	40	27	31	29	16	15	17	17	29
Manitoba	Dauphin	34	39	39	44	47	26	26	20	16	18	19	24	29
	Robbin	29	38	38	44	40	27	24	17	17	18	20	21	28
	Winkler	34	38	36	39	46	38	23	19	21	19	15	24	29
	Melita	29	37	44	44	41	33	26	26	25	26	23	32	32
Saskatchewan	Saskatoon	38	40	44	43	48	34	26	29	24	24	28	33	34
	Yorkton	38	38	39	47	49	28	24	23	20	20	22	28	32
	Norquay	34	40	38	42	42	26	21	19	14	10	16	25	27
	Moose Jaw	33	42	44	49	48	33	28	22	22	16	21	—	32
Alberta	Tofield	33	39	41	41	49	29	27	18	21	20	19	22	30
	Sedgewick	34	42	43	44	40	30	26	26	24	27	27	30	33
	South Edmonton	35	34	33	35	40	31	25	19	21	18	23	20	28
	Wetaskiwin	32	36	36	36	40	32	25	16	20	23	23	22	28
Brit. Columbia	Salmon Arm	—	34	41	39	37	34	24	19	16	18	25	34	30
	Sardis	—	—	36	35	36	38	20	19	18	19	24	37	29
Average by months		34	40	42	42	42	31	27	21	19	19	21	26	

[a] Where a monthly value is missing, the mean of adjacent months was substituted in order to calculate the "average by towns."

In addition, the results of this survey are plotted in Figure 1, with the heavy line joining the monthly means and the broken lines representing the 95% confidence limits for each month.

The data in Figure 1 indicate a wide seasonal variation between the tocopherol content of summer and winter butterfat. For July, August, September, and October, 1953, the mean tocopherol contents in parts per million for summer butterfats were 40, 42, 42, and 42, respectively, with a range of 33 to 50. For

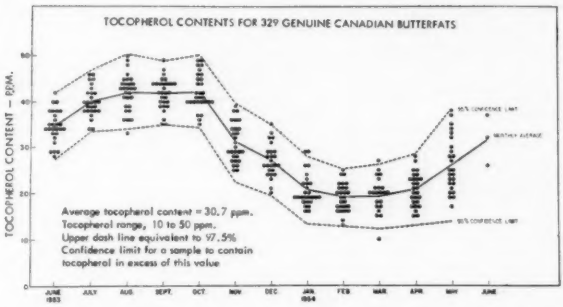

FIG. 1. Tocopherol content (p.p.m.) for 329 Canadian commercial butterfats.

January, February, March, and April, 1954 ("winter" months), the mean tocopherol contents for butterfats were 21, 19, 19, and 21 p.p.m., respectively, with a range of 10 to 29. During the "transition" months of May, 1954, and June, November, and December, 1953, the mean tocopherol contents were 26, 34, 31, and 27, respectively, as these mean contents fluctuated seasonally between the 19 to 21 "winter" levels and the 40 to 42 "summer" (July, August, September, and October) levels. In no case was a tocopherol content found higher than 50 or lower than 10 p.p.m.

The data in Figure 1 also indicate a difference of 5 p.p.m. of tocopherol between the lower 95% confidence limits for summer butterfat and the upper 95% confidence limits for winter butterfat. Therefore, the tocopherol contents of summer and winter butterfats are significantly different, indicating the important effect of season upon the tocopherol content.

In order to combat the adulteration of butter with vegetable fats, the Canadian Food and Drug Regulations *(2)* were amended *(3)* by the insertion of a clause to the effect that "Milk Fat (Butter Fat) . . . shall have . . . a tocopherol content not greater than 50 micrograms per gram as determined by the official methods." Since the Food and Drug Regulations *(3)* impose only an upper limit on the tocopherol content of butterfat, interest was focused on determining the statistical chances of the tocopherol content of unadulterated butterfat exceeding the upper limit of 50. Therefore, the data in Table 1 were analyzed statistically to determine the tocopherol contents corresponding to the upper confidence limits for $P = 0.05$, 0.01, and 0.0005. These values are given in Table 2.

The data in Table 2 indicate that for summer butterfat, 95% of unadulterated samples should have tocopherol contents below 49, 99% below 52, and 99.95% below 57 p.p.m. For winter butterfats, 95% of samples should be below 27, 99% below 30, and 99.95% below 35 p.p.m. Therefore, there is only a slight possibility that the tocopherol content of genuine butterfat will exceed 50 p.p.m. and then only during the summer months.

TABLE 2

Tocopherol contents corresponding to the upper confidence limits for probabilities of 0.05, 0.01, and 0.0005

Month of butterfat production		Tocopherol contents in p.p.m. corresponding to the upper confidence limit for		
		P = 0.05	P = 0.01	P = 0.0005
June	1953	40.7	43.5	47.9
July	Summer	45.8	48.3	52.4
August	Summer	49.0	52.0	56.9
September	Summer	47.7	50.3	54.4
October	Summer	48.9	51.8	56.9
November		38.2	41.5	46.6
December		33.5	36.5	41.2
January	1954	26.7	29.4	33.8
February	Winter	24.3	26.6	30.3
March	Winter	25.1	27.7	31.9
April	Winter	27.3	29.8	34.9
May		36.5	41.2	48.7

DISCUSSION

Practical application of the tocopherol procedure to the detection of butterfat adulteration. The tocopherol procedure *(5)* has been successfully employed in Canada for 18 months for the detection of butterfat adulteration with vegetable oils other than coconut. To illustrate the type of results that have been obtained, some of the tocopherol contents for 127 butter churnings (850 to 1,900 lb. butter per churning in this case) from a single butter manufacturer are given in Figure 2. The lower heavy line in the figure represents the mean tocopherol contents for May and June, the months these butters were produced, and the two broken lines are spaced ±10 p.p.m. tocopherol above and below this line corresponding approximately to the over-all 95% confidence limits for May and June butterfats.

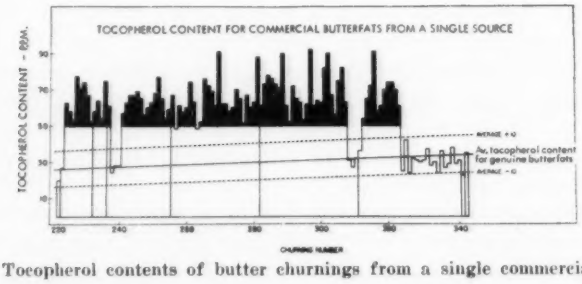

FIG. 2. Tocopherol contents of butter churnings from a single commercial source.

These data indicate that a large number of these butterfats contained tocopherol far in excess of the normal range for butterfat produced in May and June. However, the tocopherol contents for butter churnings 324 to 343 appear normal and represent butter production immediately after the seizure of over 50 tons of butter from a storage warehouse. This sudden change in tocopherol content indicates the prompt cessation of adulteration.

It was difficult to understand why the tocopherol contents for these adulterated butterfats should continue to fluctuate from 52 to 92 p.p.m. even after the practice of adulteration should have become routine. It was later discovered that if the tocopherol contents for the adulterated butterfats were plotted against the amount of butter per churning, a good correlation was obtained, as indicated in Figure 3.

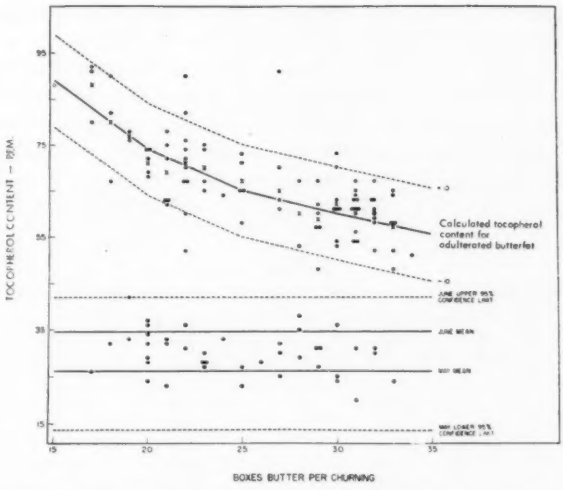

FIG. 3. Correlation between tocopherol contents and churning size for 127 churnings of commercial butter.

In Figure 3 the group of points distributed horizontally across the bottom of the figure is considered to belong to unadulterated butterfats because all these points fall between the 95% confidence limits for the tocopherol content of unadulterated May and June butterfats. In the other group of points, the tocopherol contents decrease with increased size of the butter churning and are considered to belong to adulterated butterfats.

The mean tocopherol content for May and June genuine butterfats is 30 (Table 1). The adulterated samples had an average tocopherol content of approximately 60 p.p.m. in a churning of 30 boxes, and this value increased to approximately 75 p.p.m. when the churning size was 20 boxes (Fig. 3). Calculations show that such a change in tocopherol content could result from the addition of one box of adulterant with a tocopherol content of 960, for example,

a commercial shortening *(5)*; two boxes of adulterant with a tocopherol content of 510 p.p.m. or some other comparable adulterant per churning of butter. From this observation, it was possible to calculate the expected tocopherol content for adulterated butterfats corresponding to 15 to 35 boxes of butter per churning. The upper heavy line in Figure 3 represents these calculated contents and the broken lines are spaced ±10 p.p.m. of tocopherol above and below this line, corresponding approximately to the over-all 95% confidence limits for May and June butterfats. The mean tocopherol contents for the adulterated butterfats corresponding to each number of boxes of butter per churning are indicated by crosses. These crosses fall very close to the calculated tocopherol line, and in addition 95% of the points for the adulterated butterfats fall within ±10 p.p.m. of the calculated tocopherol line. These results tend to confirm the belief that in this case a fixed amount of adulterant had been added routinely to the widely variable amounts of butter per churning; hence the very variable amounts of tocopherol found in these adulterated butterfats.

Other adulterators appeared to have added a fixed percentage of adulterant to their butter, resulting in elevated but more or less uniform tocopherol contents for the adulterated butterfats. Tocopherol contents as high as 480 have been encountered in commercial butterfat and as high as 168 p.p.m. in ice cream fat.

Some of the butterfat samples shown in Figures 2 and 3 were also analyzed by the Reichert-Meissl and Polenske procedures with the results given in Table 3.

TABLE 3

Reichert-Meissl values, Polenske values, and tocopherol contents for six adulterated butterfats

Sample No.	Reichert-Meissl value	Polenske value	Tocopherol content
			(p.p.m.)
1	30.2	1.7	61
2	29.6	1.8	74
3	29.3	1.8	61
4	29.4	1.6	91
5	30.1	1.7	82
6	29.9	1.7	90
Food & drug limits for genuine butterfat *(3)*	Min. 24.0	Max. 3.5 or 10% of R.M.	Max. 50

The data in Table 3 show that with the Reichert-Meissl and Polenske procedures it was not possible to detect the adulteration of these butterfats; however, the tocopherol values clearly indicated adulteration. Such results emphasize the value of the tocopherol procedure for detecting the adulteration of butterfat with small amounts of vegetable fats (except coconut).

SUMMARY

Tocopherol contents have been determined for 329 Canadian commercial butterfats in a 1-year survey. The monthly mean tocopherol contents in parts per million are: June, 34; July, 40; August, 42; September, 42; October, 42;

November, 31; December, 27; January, 21; February, 19; March, 19; April, 21; and May, 26. The over-all tocopherol range was 10 to 50 with a mean content of 31.

Confidence limits indicate that for butterfats produced in July, August, September, and October, 95% of unadulterated samples should have tocopherol contents below 49; 99% below 52; and 99.95% below 57 p.p.m. For butterfats produced in January, February, March, and April, 95% of samples should be below 27; 99% below 30; and 99.95% below 35 p.p.m. Confidence limits for other months fall intermediate between the summer and winter levels.

An example of the use of the tocopherol determination for the detection of butter adulteration is given.

ACKNOWLEDGMENT

The authors wish to express their thanks to J. C. Woodward, A. H. White, and R. Riel of the Science Service, Canada Department of Agriculture, for supplying the samples of butterfat employed in this survey.

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FACTORS NECESSARY FOR THE GROWTH OF *BACTEROIDES SUCCINOGENES* IN THE VOLATILE ACID FRACTION OF RUMEN FLUID

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Studies on the kinds of bacteria present in the bovine rumen have shown that *Bacteroides succinogenes* is one of the more important cellulolytic agents therein *(6, 16)*. Work on the growth requirements of this species has shown that rumen fluid contains a growth factor that was not detectable in several materials commonly used to grow nutritionally exacting bacteria or in extracts from alfalfa meal or bovine feces *(6)*. This factor was found to be stable in rumen fluid that was autoclaved at pH 3 and pH 9 for 1 hour at 15 lb. pressure and in rumen fluid dried on a steam bath and reconstituted with distilled water. It was not replaced by known B vitamins, minerals, several purines and pyrimidines, oleic acid, acetic acid, Tween 80, or an acid or enzymatic hydrolyzate of casein, although some of these substances undoubtedly are also required. Hungate *(16)* concluded that the factors required for growth of this organism are not all present in the feed consumed by the animal but are in part synthesized by indigenous ruminal microorganisms.

The purpose of the present work was to determine the nature of this unknown factor(s).

EXPERIMENTAL PROCEDURE

The general procedure followed in determining the identity of the unknown factor was to prepare a complex medium of known composition except for "vitamin free" casein hydrolyzate and assume that this medium contained all factors required for the growth of the organism except the unidentified factor in rumen fluid. This medium was then used to assay fractions of rumen fluid obtained by ether extraction, steam distillation, and partition chromatography. These studies indicated that the active factor was present in the volatile acid fraction of rumen fluid (see below), and, because of the difficulty of separating the volatile acid isomers, commercially available volatile acids were assayed to further identify the active factor.

The composition of the assay medium is shown in Table 1. Initially, cellulose was used as substrate, but glucose was later substituted so that faster and more accurate turbidimetric measurements of growth could be made. One hundred ml. of glucose medium was prepared as follows: 7.5 ml. 0.6% K_2HPO_4 solution; 7.5 ml.

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TABLE 1
Composition of assay medium

	(Mg/100 ml.)		*(Mg/100 ml.)*
Thiamin · HCl	0.2	Glucose or cellulose[b]	300
Pyridoxamine · 2HCl	0.05	Cysteine · HCl	50
Pyridoxal · HCl	0.05	Na_2CO_3	400
Ca-D-pantothenate	0.2	K_2HPO_4	45
Riboflavin	0.2	KH_2PO_4	45
Nicotinamide	0.2	NaCl	90
p-Aminobenzoic acid	0.01	$(NH_4)_2SO_4$	90
Biotin	0.005	$CaCl_2$	9
Folic acid	0.005	$MgSO_4$	9
Cobalamin	0.0005	$FeSO_4 \cdot 7H_2O$	4
Casein hydrolyzate[a]	200	$ZnSO_4$	0.15
Adenine · $SO_4 \cdot 2H_2O$	0.5	$CuSO_4 \cdot 5H_2O$	0.15
Guanine · HCl	0.5	$NaMoO_4 \cdot 2H_2O$	0.15
Uracil	0.5	$CoCl_2 \cdot 6H_2O$	0.15
Xanthine	0.5	Resazurin	0.1
Thymine	0.5		

[a] Nutritional Biochemicals Corporation "vitamin-free" casein hydrolyzate (enzymatic).
[b] Cellulose was in the form of Whatman No. 1 filter paper ground in a ball mill 72 hr. in a 2% water suspension.

solution of 0.6% KH_2PO_4, 1.2% NaCl, 1.2% $(NH_4)_2SO_4$, 0.12% $CaCl_2$ and 0.12% $MgSO_4$; 1 ml. 100 × vitamin mixture, 1 ml. 100 × purine-pyrimidine mixture, 1 ml. 100 × micro-mineral mixture, 0.1 ml. 0.1% resazurin solution, 4 ml. casein hydrolyzate (5% solids), 0.3 g. glucose, and 60 ml. of distilled water were placed in a flask and adjusted to pH 6.7 with 10% sodium hydroxide. The medium was boiled under carbon dioxide gas and the flask was then closed with a rubber stopper. After the medium was autoclaved at 15 lb. for 15 minutes, it was cooled, and sterile solutions of 3% cysteine hydrochloride (1.7 ml.) and 6% sodium carbonate (6.7 ml.) were added. The medium was thoroughly mixed and tubed under carbon dioxide in 9-ml. amounts in sterile, rubber-stoppered, 16 × 150 mm. culture tubes by using the anaerobic procedure outlined previously *(5)*. One ml. of the assay material brought the constituents in the medium to final concentration.

In preliminary experiments the inoculum consisted of 0.1 ml. of an actively growing 24-hour culture of *Bacteroides succinogenes*, strain S-85, in a 20% (v/v) rumen fluid–cellulose medium. After it was found that the volatile acid fraction of rumen fluid would support growth in the basal medium (see below), the inoculum consisted of 0.1 ml. of an 18-24 hour culture in the basal glucose medium with 1.4 meq. of volatile acid from rumen fluid per 100 ml. added. This amount of volatile acid was approximately equal to the amount present in a 10% rumen fluid medium.

The organism was maintained in RGCA slant medium *(5)* in a "dry ice" box, and three to four daily transfers were made in the inoculum medium before assays were made.

Growth in cellulose medium was estimated by visible loss of cellulose from the tube, and growth in glucose medium was estimated in 18-20 hour cultures (see Figure 1) by using a Cenco-Sheard spectrophotelometer with an 11-mm. cell and wave length of 420 mμ.

Rumen fluid used in fractionation experiments was collected and prepared

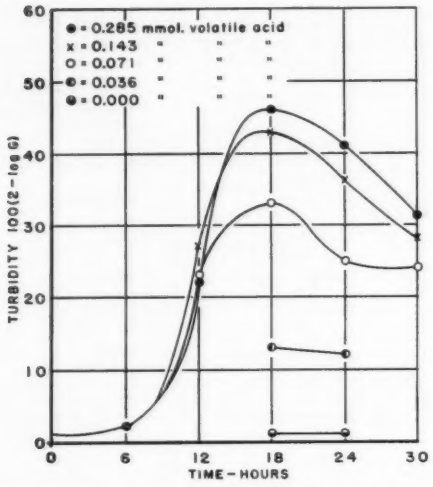

FIG. 1. Growth on glucose basal medium with volatile acid from rumen fluid added.

for use as previously described *(5)*. Ether extracts were made by wet extraction for 24 hours with a Soxhlet apparatus. Extractions were made at pH 2 and pH 10. The extracts and residues were adjusted to pH 10 with sodium hydroxide or sulfuric acid, evaporated to dryness on a steam bath, and reconstituted to proper volume with distilled water. Rumen fluid was steam distilled at pH 2.0 with an apparatus similar to that of Friedemann *(10)*. The distillate and residue were neutralized to pH 10, evaporated on the steam bath, and reconstituted with distilled water. Volatile acids from rumen fluid were fractionated by using the chromatographic procedure of Moyle *et al. (18)*, in which phosphate buffered silicic acid columns and three different solvents, namely, 1% *n*-butanol in chloroform (CB_1), CB_{10}, and CB_{30}, are used. Column I was prepared with five times the cross-sectional area of Moyle's Column I to effect the separation of large amounts of acetic, propionic, and butyric acids so that measurable amounts of the higher acids would be available for separation on Column III.

Fatty acids assayed included sodium acetate, A.C.S.; *n*-caproic acid and sodium propionate, C.P.; *n*-heptanoic acid and *n*-caprylic acid, purified; DL-α-methyl-*n*-butyric acid, highest purity; and isobutyric acid, reagent grade;[1] isocaproic, trimethylacetic, *n*-valeric, isovaleric, lauric, palmitic, and stearic acids;[2] and sodium butyrate, pure.[3] The free acids were neutralized with sodium hydroxide before use. All substances assayed were diluted to various concentrations in distilled water and sterilized in the autoclave before they were added to the sterile basal medium.

[1] Fisher Scientific Company.
[2] Eastman Kodak Company.
[3] Amend Drug and Chemical Company.

Surface tension measurements of culture media and rumen fluid were made with a du Nouy tensiometer. The measurements were made in triplicate on each sample, and two samples of each medium were used. The readings were made at 25° C. and completed as fast as possible to eliminate large changes in the carbon dioxide content of the media on exposure to air. The rumen fluid measurements were made after passage of the fluid through a Seitz filter and saturation with carbon dioxide.

RESULTS

Table 2 shows the results of the experiment on ether extraction and steam distillation fractionation procedures. The basal medium allowed no appreciable growth unless rumen fluid or active fraction of rumen fluid was added. The factor was found in the acid ether extract but not in the residue. Most of the activity in the alkaline ether extraction was found in the residue. The small amount of activity in the alkaline extract at the 20% level was undoubtedly due to emulsification, which allowed a small amount of the residue to be carried over in the extract. When the extracts and residues were recombined, growth was good. This indicated that fractions not allowing growth were not toxic. These results plus the data showing the factor to be present in the steam distillate suggested that the factor was one, or a combination, of the volatile fatty acids present in the rumen fluid.

Figure 1 shows the growth curve obtained when the volatile acid fraction of rumen fluid was added to the basal medium containing glucose as substrate. Maximum turbidity occurred at about 18 hours with a subsequent rapid drop.

TABLE 2

The occurrence of the rumen fluid factor in fractions of rumen fluid obtained by ether extraction and steam distillation

Fraction added to basal medium	Concentration[a]	Growth[b]
	(%)	
Distilled water		−
Rumen fluid	5	++
Rumen fluid	20	++++
Ether extract pH 2	5	++
Ether extract pH 2	20	++++
Residue after ether extraction pH 2	5	−
Residue after ether extraction pH 2	20	−
Ether extract + residue pH 2	20	++++
Ether extract pH 10	5	−
Ether extract pH 10	20	±
Residue after ether extraction pH 10	5	++
Residue after ether extraction pH 10	20	++++
Ether extract + residue pH 10	20	++++
Steam distillate pH 2	5	++
Steam distillate pH 2	20	++++
Residue after steam distillation pH 2	5	−
Residue after steam distillation pH 2	20	−
Steam distillate + residue	20	++++

[a] The concentration is expressed as the equivalent amount of that fraction in a 5 or 20% rumen fluid medium.

[b] − = no loss of cellulose after 4 days on first transfer.
++++ = all cellulose gone after 24-hr. incubation of fourth daily transfer.

Microscopic examination of old cultures revealed mostly swollen cells and cellular debris, indicating rapid lysis on aging.

Surface tension measurements of the basal medium, basal medium plus volatile acid from rumen fluid, and rumen fluid showed no appreciable differences (63-64 dynes per centimeter at 25° C.) and suggested that interfacial phenomena were not involved.

The individual volatile acids and their molecular percentage composition of total volatile acid (5.6 m*M* from 40 ml. rumen fluid) as obtained by the chromatographic technique were as follows: acetic, 70.8; propionic, 14; butyric, 13; valeric, 1.7; caproic, 0.43; and heptanoic and/or higher, 0.11. The individual fractions, with the exception of acetic acid, were assayed in the glucose basal medium. The valeric acid fraction was very active, 9 μM allowing good growth, whereas little activity was present in the propionic acid fraction. No activity was present in the other fractions.

Because of the difficulty of fractionating valeric acid isomers from rumen fluid, the next experiment was to assay commercially available valeric acid isomers as well as other volatile fatty acids. Figure 2 shows some of the results

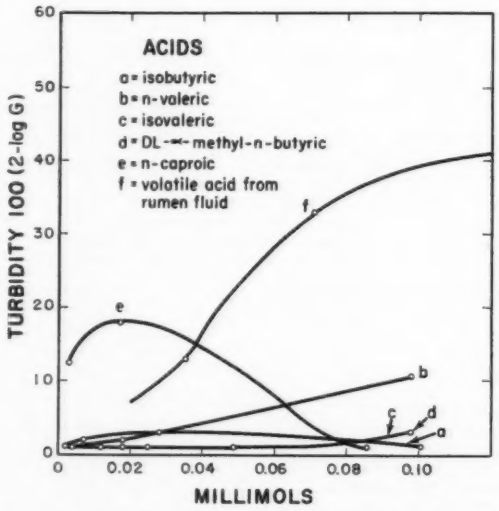

FIG. 2. Growth on glucose basal medium with commercially available volatile fatty acids added.

of this work. *n*-Caproic acid exhibited about half-maximum activity in low concentrations but was inhibitory at higher levels. *n*-Valeric, isovaleric, DL-α-methyl-*n*-butyric, and isobutyric acids showed very low activity. Acetic, propionic, *n*-butyric, trimethyl acetic, isocaproic, *n*-heptanoic, and *n*-caprylic acids showed no activity.

The type of curve obtained with the *n*-caproic acid suggested that it might contain two active factors, one of which replaced the rumen fluid factor and

another which was inhibitory to growth. The *n*-caproic acid was run through Column III of Moyle *et al. (18)*. It appeared to contain a contaminating acid which passed through the column in a position similar to valeric acid isomers. This contaminant was estimated to be 7.1% and 7.7% of the total acid in two separate runs. Neither the caproic fraction nor its contaminant ("valeric" acid) allowed growth comparable to that obtained with the unfractionated acid. However, when the two fractions were combined, the growth response was similar but somewhat better than that with the original unfractionated acid (Figure 3). These results suggested that mixtures of volatile acids might be required.

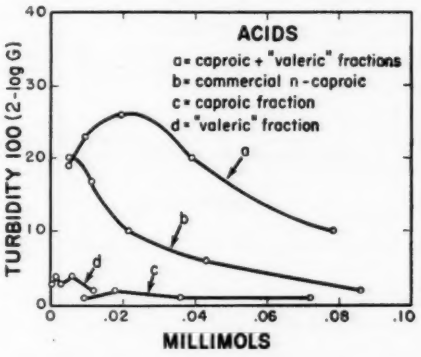

FIG. 3. Growth on glucose basal medium with commercially available *n*-caproic acid and its chromatographically separated fractions.

Mixtures similar to the acids found in rumen fluid were prepared from the commercial volatile acids. Mixture 1 contained 61% acetic (molecular basis); 20% propionic; 10% *n*-butyric; 2% isobutyric, *n*-valeric, isovaleric, and DL-*a*-methyl-*n*-butyric and 1% of *n*-caproic acid. Mixture 2 was Mixture 1 minus the acetic, propionic, and *n*-butyric acid. Mixture 3 contained only the valeric isomers. These mixtures were added to the basal medium in amounts representing 40, 20, 10, and 5% rumen fluid media, assuming rumen fluid to contain 15 m*M* of volatile acid per 100 ml. All three mixtures allowed good growth at the 10% level, and Mixtures 1 and 2, at the 5% level. Mixture 1 showed inhibition at the 40 and 20% levels, and Mixture 3 showed a lower level of growth at the 5% level. This suggested that the acids in Mixture 2 might all be concerned in the activity of the volatile acids.

With this in mind, assays were made with all possible combinations of two and of three of these acids. Data in Figure 4 show that only the solutions containing *n*-valeric or *n*-caproic acid in combination with any one of the branched-chain acids, isobutyric, isovaleric, or DL-*a*-methyl-butyric acid, allowed a significant amount of growth. Combinations of branched-chain acids allowed no growth, and the combination of *n*-acids allowed only slight growth. This slight growth was probably due to the contaminant in the *n*-caproic acid. As

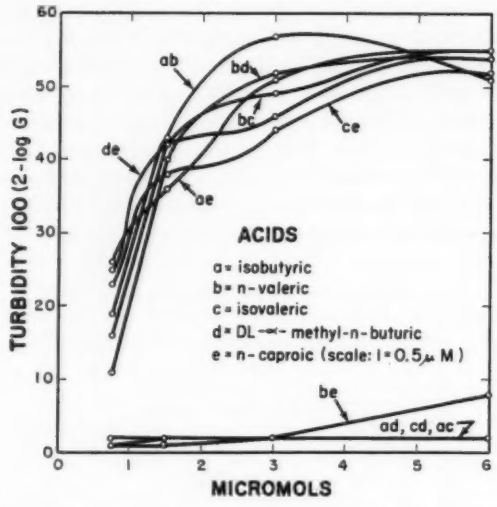

FIG. 4. Growth on glucose basal medium with combinations of two volatile fatty acids.

might be expected, all combinations of three acids allowed good growth with the exception of the one combination which did not contain an *n*-acid (Figure 5).

To see if other acids might replace *n*-valeric or *n*-caproic, various acids were assayed in the presence of isobutyric and isovaleric acid. Concentrations used

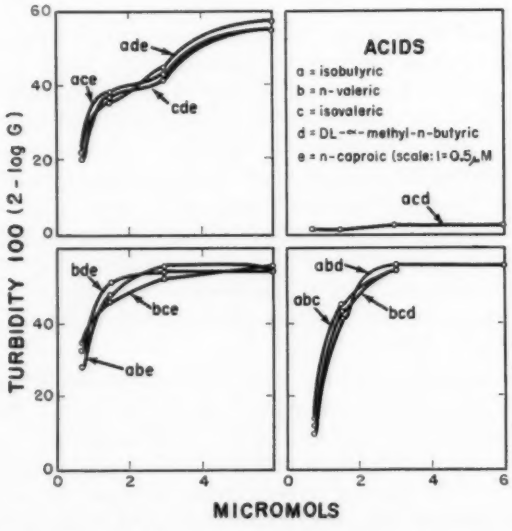

FIG. 5. Growth on glucose basal medium with combinations of three volatile fatty acids.

were 6, 3, 1.5, and 0.75 μM of each. *n*-Heptanoic and *n*-caprylic acid allowed growth comparable to *n*-valeric and *n*-caproic, whereas *n*-butyric, propionic, acetic, isocaproic, trimethyl acetic, or lauric acid did not. Palmitic acid allowed approximately half-maximum growth, and stearic acid, slight growth.

To determine whether other acids could be substituted for the branched-chain acids, isobutyric, isovaleric, or DL-*a*-methyl-*n*-butyric, various acids were tested in the presence of 6 μM of *n*-valeric acid. Acetic, propionic and *n*-butyric acid were tested at concentrations of 400, 100, 25, 6.3, and 1.6 μM; and other acids at 25, 6.3, 1.6, and 0.4 μM. Acetic, *n*-butyric, isocaproic, *n*-heptanoic, *n*-caprylic, and trimethyl acetic acid allowed no growth, and propionic acid allowed light growth only when 400 μM were added and none at the lower concentrations. Isocaproic acid did not replace the active branched-chain acids when *n*-heptanoic acid was substituted for *n*-valeric acid.

TABLE 3

Effects of various concentrations of n-*valeric acid and isovaleric acid on the growth of* Bact. succinogenes

	100(2 − log G)				
	Valeric acid (μM)				
Isovaleric acid (μM)	6	3	1.5	0.75	0.38
6	49	44	30	8	2
3	44	42	14	3	1
1.5	48	43	12	3	1
0.75	43	32	15	3	1
0.38	24	16	6	2	1
0.19	8	9	3	1	1
0.094	4	7	3	1	1
0	1	1	1	1	1

Table 3 shows the effect of varying the concentrations of *n*-valeric and isovaleric acid on growth of the organism. At minimal concentrations for good growth the ratio of *n*-valeric acid to isovaleric acid was about 2:1.

The activity of the mixture of acids, isobutyric, *n*-valeric, isovaleric, DL-*a*-methyl-*n*-butyric, and *n*-caproic, was identical whether they were sterilized in the autoclave or by filtration through a sintered glass filter.

All available strains of *Bact. succinogenes* grew well on the basal medium with Mixture 2 added but not in the basal medium alone. The strains studied were S61, S85, S111, S121, M13, and M34, described previously, and a newly isolated strain. The former strains showed some differences in pigment production, morphology, and sugars fermented *(6)*.

DISCUSSION

The results show that two fatty acid components are necessary for growth of *Bact. succinogenes*. One of these components is a branched-chain volatile acid, which may be either isobutyric, isovaleric, or DL-*a*-methyl-*n*-butyric acid. The other component is a straight-chain acid. Any one of the C_5 to C_8 *n*-acids will

satisfy this requirement. Whether higher acids can be used was not completely determined. Stearic and palmitic acid allowed only a small amount of growth, probably because of their poor solubility in the basal medium. The failure of lauric acid to substitute for other *n*-acids is probably best explained by the known bacteriostatic effects of C_8 to C_{14} saturated fatty acids *(14)*.

The major contributors to these two components in rumen fluid are probably isobutyric, isovaleric, and D-α-methyl-*n*-butyric acids for the branched-chain member, and *n*-valeric and *n*-caproic acids for the straight-chain member. All of these acids have been shown to be present in rumen fluid *(1, 8)*. The probable origin of the acids by Stickland reactions from amino acids has been discussed by El-Shazly *(9)*.

That acetate *(13)* and long chain unsaturated fatty acids *(19)* are necessary for the growth of various bacteria has been known for some time. A pronounced synergistic growth effect was demonstrated in *Lactobacillus arabinosus* between various saturated *n*-fatty acids and unsaturated fatty acids *(2)*. However, the seemingly obligate requirement of *Bact. succinogenes* for two saturated fatty acids, one an *n*-acid and the other a branched-chain acid, is unique among organisms studied heretofore and suggests that this microorganism, growing in the presence of relatively high concentrations of higher volatile fatty acids in rumen fluid, may have lost its ability to synthesize some unknown, long, branched-chain acid(s) from smaller carbon units. The occurrence of branched-chain fatty acids in certain bacteria is well known *(7)*. Also of interest are reports of the occurrence of long branched-chain saturated fatty acids in wool fat, butterfat, beef fat, and mutton fat *(15)*. These acids include C_{10} to C_{28} iso acids and C_9 to C_{31} anteiso acids and a multibranched C_{20} acid. The branched-chain acid requirement of *Bact. succinogenes* is met by either iso or anteiso acids.

Another possible reason for the two-component requirement is that one of the components is required but inhibitory and the other component neutralizes the former's inhibitory effect. A similar phenomenon has been shown with amino acids *(12)*.

The possibility that other bacteria might require these fatty acids is of interest. Several other kinds of rumen bacteria have been isolated that will not grow, or grow feebly, in a yeast extract–Trypticase medium but grow well if rumen fluid is added *(5)*. A similar phenomenon was observed by McNeill *et al. (17)*. Sijpesteijn *(21)* found that *Ruminococcus flavefaciens* grew much better in a medium containing a sterile filtrate of a medium in which *Clostridium sporogenes* had been grown. This organism undoubtedly produced various volatile fatty acids from amino acids in the medium and these may have been responsible for the improved growth of *R. flavefaciens*.

A long chain, saturated, low-melting point fatty acid present in butterfat *(3, 11)* has been shown to be stimulatory to rats fed certain rations *(4)*. One postulate advanced was that butterfat allowed the intestinal microflora to develop that in turn produced the factors needed by the animal *(3)*. It is possible that this phenomenon is related to findings of the present study.

The present findings add considerably to the knowledge of the nutrition of

an important cellulolytic bacterium of the rumen and should make it a relatively simple task to establish the nutritional requirements of the species, which in turn may lead to a better understanding of cellulose digestion and fatty acid metabolism.

SUMMARY

Factors necessary for the growth of *Bacteroides succinogenes* and present in the volatile acid fraction of rumen fluid have been identified. Any one of the branched-chain saturated acids, isobutyric, isovaleric, or DL-*a*-methyl-*n*-butyric acid can be used as one component. Any one of a number of straight-chain saturated fatty acids can be used as the second component. These include C_5 to C_8 acids. Some activity for the second component was found in stearic and palmitic acid but none in lauric acid. Minimum concentrations for good growth when *n*-valeric and isovaleric acids were used were about 3 and 1.5 μM, respectively, per 10 ml. of medium. All available strains of *Bact. succinogenes* require these acids.

After this work was submitted for publication, Bentley *et al.* reported that the volatile acids, *n*-valeric, isovaleric, isobutyric, and caproic, or their amino acid precursors, stimulated cellulose digestion and the conversion of urea nitrogen into protein by rumen microorganisms as measured by the artificial rumen technique (*J. Am. Chem. Soc.*, 76: 5000. 1954).

ACKNOWLEDGMENTS

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HEAT DENATURATION OF THE SPECIFIC SERUM PROTEINS IN MILK

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Many studies have shown the effect of heat treatment on the serum (whey) proteins in milk. These proteins when denatured by heat are removed with the casein when precipitated at its isoelectric pH, by saturated sodium chloride, or by various other procedures. Rowland *(16, 17)*, Harland *et al. (5, 6)*, Larson *et al. (10)*, and others *(7, 13, 18)* have demonstrated that the serum proteins are denatured according to the extent of the heat treatment under the conditions used for the commercial processing of milk and milk products. All of these studies have been concerned, however, with either the denaturation of the total or the "globulin" and "lactalbumin" fractions of the serum proteins.

The classical "globulin" fraction, which is the highest in colostrum, is probably identical with or closely related to some of the immune globulins of blood serum *(12, 19, 20)*. The classical "lactalbumin" fraction has been shown to contain the proteins α-lactalbumin, β-lactoglobulin, and serum albumin, all of which have been crystallized from milk *(4, 12, 14, 15)*. Of these three proteins, apparently only serum albumin is identical with any of the blood proteins and is the same as the serum albumin of blood *(3, 15)*. In addition, several enzymes and probably other proteins are present in the serum protein fraction of milk *(12)*; however, on a quantitative basis their contribution to the total protein content is small.

Although studies have been conducted on the heat denaturation of several of these milk proteins as the purified systems isolated from milk and blood, the heat denaturation of the specific serum proteins of the "lactalbumin" fraction have not been studied individually as they exist in milk. For this reason, samples of a single lot of milk were given various heat treatments, and the contents of the specific proteins remaining undenatured in the serum were evaluated by a quantitative electrophoretic procedure.

EXPERIMENTAL

A sample of mixed milk was obtained from the University herd soon after milking and centrifuged at 2000 × G. for 20 minutes at 40° C. The skimmilk (pH 6.65) was divided into 125-ml. portions, which were individually heat-treated the following day at 40° (warmed only for centrifuging and considered as the original milk), 56°, 62°, 66°, 70°, 74°, 77°, 82°, and 96° C. for 30 minutes with slow agitation in a 500-ml. round bottom flask equipped with a condenser and thermometer. Five minutes was allowed for the milk to come to the water bath temperature. After heating, the flask was quickly cooled, and 20 ml. of milk was removed for analysis of the total undenatured serum proteins and nonprotein nitrogen according to the procedures of Rowland *(17)*. In this study the un-

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denatured serum proteins were taken as the protein remaining in the serum after removal of the heat denatured serum proteins with the casein and correction made for the nonprotein nitrogen present.

The remaining heated milk was brought to pH 4.6 by the slow addition of a measured amount of 10% (by volume) acetic acid, and the casein and denatured serum proteins were removed by filtration on Whatman No. 1 paper. The volume of the clear filtrate was measured, dialyzed (24/32-in. Visking tubing) against about six volumes of water at 4° C. for 12 hours, removed from the bag, and centrifuged at 2000 × G. for 15 minutes to remove a few milligrams of protein which precipitated during the dialysis, placed back in the same size dialysis bag with 5 ml. of toluene, and dialyzed for 4 hours against about six volumes of phosphate buffer of pH 6.9 and ionic strength of 0.1 at 4° C. (final pH of 6.6). The dialysis bags were then pervaporated in a strong current of warm air and reduced to a volume of about one quarter of the original serum in 5 hours, the solutions remaining at about 10° C. This volume was measured and an aliquot was dialyzed (18/32-in. Visking tubing) against about thirty volumes of Veronal buffer *(10)* of pH 8.6 and ionic strength of 0.1 for 12 hours at 4° C. and then against a fresh change of the same amount of cold buffer for 24 additional hours. The volume of the bags was measured before dialysis and at the time of the electrophoretic analysis to correct for any change.

All electrophoretic analyses were conducted in the Veronal buffer of pH 8.6, ionic strength of 0.1, at 1.2° C. for 5,580 seconds with a field strength of 7.8 volts per square centimeter. An American Instrument Company portable electrophoresis apparatus equipped with a 6-ml. standard analytical cell and a cylindrical lens with a rotating slit angle of 54° was used. Since the electrophoretic clarity of the patterns of the ascending were sharper than those of the descending boundaries, the areas evaluated were those of the ascending as previously described *(11)*. Mobilities, however, were calculated from the patterns of the descending boundaries.

The original milk (40° C. warming) was also analyzed by the procedures of Rowland *(17)* to evaluate the total protein, casein (N precipitated at pH 4.6), total serum protein (N in pH 4.6 filtrate minus nonprotein N), "globulin" (N precipitated from pH 4.6 filtrate adjusted to pH 7.0 and saturated with magnesium sulfate), "lactalbumin" (total serum protein N minus globulin N minus proteose-peptone N), "proteose-peptone" (N in pH 4.6 filtrate from milk boiled for 20 minutes minus nonprotein N), and nonprotein N (N nonprecipitable by 12% trichloracetic acid) contents. This was done for comparative purposes with the electrophoretic analyses to evaluate the agreement between the two different analytical procedures. Protein concentrations (N × 6.38) were determined by a micro-Kjeldahl procedure after making suitable corrections when necessary for the nitrogen due to the Veronal buffer.

RESULTS

The analysis of the unheated (40° C.) milk by the Rowland *(17)* procedure is shown in Table 1 and the distribution of the proteins into the various fractions

agrees well with previous published work *(13, 17)*. The analysis of the classical "globulin," "lactalbumin," and "proteose-peptone" fractions by the electrophoretic isolation procedure agrees well with the Rowland distribution and indicates that the immune globulins account for the "globulin" fraction. This

TABLE 1

Protein analysis of original milk by the Rowland (16) and electrophoretic procedures

Fraction	Component in Fig. 1	Rowland analysis	Serum protein analysis	
			Rowland procedure	Electrophoretic procedure
		(g/100 ml.)	*(% of serum proteins)*	
Total protein[a]		3.08		
Casein		2.30		
Serum proteins		0.546	100.0	100.0
Immune globulins	1, 2	0.083	15.2	13.0
Lactalbumin		0.361	66.1	68.1
α-Lactalbumin	4			19.7
β-Lactoglobulin	6			43.7
Serum albumin	7			4.7
Proteose-peptone		0.102	18.7	18.9
Component	3			4.6
Component	5			8.6
Component	8			5.7
Nonprotein N[a]		0.232		

[a] Nonprotein nitrogen calculated as protein (N × 6.38) and is included in the total protein figure.

agreement may be somewhat fortuitous since Smith *(19)* has shown that the classical procedures for preparing milk serum globulins by precipitation with saturated magnesium sulfate or one-half saturated ammonium sulfate precipitates a complex of material. However, these conditions do remove from milk serum chiefly the electrophoretic fractions with a mobility of –1.7 and –2.4 and these fractions have been shown to contain most of the immune properties *(19)*. For this reason, the classical "globulin" fraction will be hereafter referred to as the immune globulins. The summation of the α-lactalbumin, β-lactoglobulin, and serum albumin contents account for the "lactalbumin" fraction. The amounts of these proteins present also agree well with previous reports of their contents in normal milk serum *(4, 12, 14, 15)*. In addition, the "proteose-peptone" fraction has been broken into three fractions, the basis for which will be discussed later.

The total amounts of the serum proteins remaining in solution after precipitation of the casein and denatured serum proteins are reported in Table 2 for the heated milks. It is to be noted that the amount of "undenatured" serum proteins in the sample heated at 96° C. for 30 minutes is quantitatively the same as the "proteose-peptone" fraction of the Rowland analysis (Table 1). This is understandable since the "proteose-peptone" fraction in the Rowland procedure is determined by boiling the milk before precipitation of the casein and is defined as the residual serum protein content.

In Table 2 is also shown a comparison of the protein recovered in the electrophoretic solutions to that which would be predicted on the basis of the Rowland

TABLE 2

Undenatured serum protein contents of heated milks and recovery in the electrophoretic analysis compared to the Rowland (17) analysis

	Undenatured serum proteins			
	Rowland analysis		Electrophoretic analysis	
30 min. heat treatment	Amount present	Compared to original	Amount recovered	Recovery compared to Rowland analysis
(° C.)	(g/100 ml. milk)	(%)	(g/100 ml. milk)	(%)
40	0.546	100.0	0.538	98.6
56	0.536	98.2	0.522	97.4
62	0.509	93.2	0.482	94.7
66	0.463	84.8	0.463	100.0
70	0.389	71.2	0.376	96.7
74	0.277	50.7	0.289	104.3
77	0.240	43.9	0.211	87.9
82	0.129	23.6	0.141	109.3
96	0.102	18.7	0.102	100.0

analysis. The recovery data reflect the volumetric difficulties inherent with the handling of the serum in so many steps before the actual electrophoretic analysis; however, good agreement is indicated.[1] From these recovery data it can be concluded: *(a)* all of the nonprotein nitrogen (0.232 g. per 100 ml. of milk) was dialyzed out in the preparative procedure, *(b)* with progressive heat treatment none of the proteins became dialyzable, and *(c)* the protein content of the serum isolated in this way is about the same as that evaluated in the Rowland procedure.

TABLE 3

Electrophoretic mobility of the proteins of milk serum prepared from milk heated at various temperatures for 30 minutes

Protein	Component in Fig. 1	Mobility[a]								
		40	56	62	66	70	74	77	82	96
Euglobulin	1	−1.7	−1.7	−1.7	−1.7	−1.7				
Pseudoglobulin	2	−2.4	−2.5	−2.5	−2.4	−2.3				
Prot.-pept. component[b]	3	−2.8	−2.9	−3.0	−2.9	−2.8	−3.0	−3.0	−3.1	−3.0
α-Lactalbumin	4	−3.6	−3.7	−3.8	−3.7	−3.7	−3.7	−3.7	−3.8	
Prot.-pept. component	5	−4.5	−4.5	−4.4	−4.3	−4.4	−4.4	−4.5	−4.5	−4.6
β-Lactoglobulin	5	−4.9	−5.1	−5.0	−5.0	−5.1	−5.1	−5.1	−5.2	
Serum albumin	7	−6.5	−6.7	−6.6	−6.5	−6.7	−6.6	−6.6		
Prot.-pept. component[c]	8	−7.8	−7.9	−7.9	−7.8	−7.9	−7.9	−7.9	−7.9	−7.9

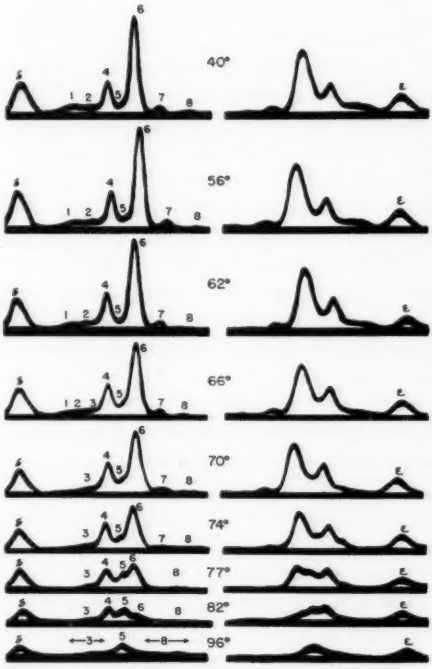
[a] Mobilities (μ) expressed in 1×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ and calculated from the descending boundary.

[b] Mobility range −1.5 to −3.2 (major material at −2.9)

[c] Mobility range −5.0 to −8.2 (major material at −7.8)

The electrophoretic patterns of the serum proteins remaining in solution after the various heat treatments and the mobility of the specific components in the solutions are shown in Figure 1 and Table 3. All of the components can be readily identified in the patterns, and no significant changes in mobility of the fractions occurred. In addition, no new components appeared in the serum

[1] Comparison of the serum protein recovery obtained in the electrophoretic analysis of over 100 unheated milk samples has shown an average of about 93% of that shown by the Rowland analysis.



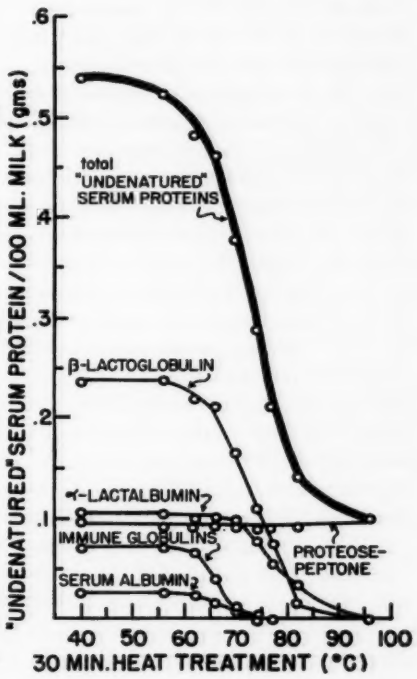


FIG. 1 FIG. 2

FIG. 1. Electrophoretic patterns of the serum isolated from milk heated at various temperatures for 30 minutes. Protein concentrations were, respectively, 1.89, 2.13, 1.85, 1.76, 1.35, 1.16, 0.85, 0.68, and 0.39%.

FIG. 2. The effect of heating milk for 30 minutes at various temperatures on the protein content of the isolated serum and the denaturation of the individual proteins present.

from the heated milks, indicating that any altered proteins, such as β-lactoglobulin which increases in mobility when heat denatured in phosphate buffer *(2)*, were removed with the casein precipitate. The amounts of the various proteins remaining undenatured at each temperature are shown in Figure 2 and have been calculated from the areas due to the components in the electrophoretic patterns.

Before such calculations could be made, however, it became evident that a correction factor needed to be applied for the material in the "proteose-peptone" fraction. It should be recognized that although this fraction has been called the "proteose-peptone" fraction *(16, 17)*, some of the material present may not be of proteose or peptone nature. Any protein not precipitated with the casein from heated milk is included in this fraction, as well as small amounts of non-precipitable degradation and interaction products that may be formed as a result of the heat treatment.

Examination of the electrophoretic pattern of the serum from the milk heated at 96° C. for 30 minutes ("proteose-peptone" fraction) revealed a major fraction (Component 5 in Figure 1), a fraction with heterogeneous electrophoretic properties and slower mobility than Component 5 (Component 3 in Figure 1), and a fraction with heterogeneous electrophoretic properties and faster mobility than Component 5 (Component 8 in Figure 1). Component 5 is partially obscured in the patterns of serum from the unheated milk and is apparent only as a small peak or an asymmetry on the side of the β-lactoglobulin peak; however, with increasing heat treatment and removal of the β-lactoglobulin it becomes more prominent. Component 3 is obscured by the immune globulins in the serum from the milk heated below 70° C. for 30 minutes; however, when the immune globulins have been removed, this fraction remains. Component 8 is partially evident in the unheated and low-heated samples as material with a mobility greater than serum albumin; however, part of it is obscured by the β-lactoglobulin and serum albumin present. Since the "proteose-peptone" fractions contribute to the total area of the other protein fractions, corrections were made in the total area of the denaturable proteins by subtracting that due to the "proteose-peptone" material. The validity of such calculations is without doubt in the case of Component 5 and part of Component 8, which can be seen in all of the patterns. Since parts of the Components 3 and 8 are completely obscured in the patterns of the serum until it has been heated, it must be assumed that they were present in the unheated milk and not formed as a result of the heat treatment. This supposition is strengthened by the observation that the corrected total "lactalbumin" fraction evaluated by the electrophoretic analysis agrees well with that evaluated by the Rowland *(17)* procedure. Even if such an assumption were incorrect, the denaturation curves for the proteins would not be appreciably changed since their total contribution is large compared to that of the "proteose-peptone" fraction.

In order to further characterize the "proteose-peptone" fraction, several samples of milk were heated for periods of 30 minutes and longer at 96° C. and the serum was concentrated by pervaporation to a greater protein concentration before conducting the electrophoretic analysis. A typical pattern is shown in Figure 3, and the three "proteose-peptone" fractions are evident. With a longer electrophoretic analysis, a further scattering of Components 3 and 8 is evident, and in some cases a tendency for Component 5 to split into several peaks was apparent.

With suitable correction made for the protein contained in the "proteose-

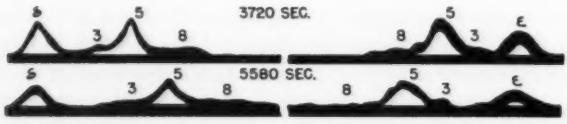


FIG. 3. Electrophoretic patterns of the proteose-peptone fraction of the serum isolated from milk heated at 96° C. for 30 minutes. The protein concentration was 0.74% and photographs of the boundaries were taken at the indicated times.

peptone'' fractions, the curves in Figure 2 demonstrate the effect of temperature on the denaturation of the serum proteins in milk. The shape of the curve and extent of the denaturation of the total serum proteins at the various temperatures is in agreement with previous work *(6, 7, 10, 16)*. It is apparent that the curve for the complete serum proteins is the composite of the denaturation curves for the individual proteins. The heat treatment of 70° C. (158.0° F.) for 30 minutes, for example, denatured 29% of the total serum proteins, and this was due to the specific denaturation of 89% of the immune globulins, 32% of the β-lactoglobulin, 52% of the serum albumin, and only 6% of the α-lactalbumin. The value for the sum of the three ''proteose-peptone'' fractions shows no change through the temperature range and constitutes the residual material of the complete serum protein denaturation curve.

It is apparent from Figure 2 that the percentage composition of the total undenatured proteins of the milk serum changes with increasing heat treatment. Since α-lactalbumin is the most heat-resistant of the denaturable serum proteins, its concentration relative to the other proteins increases up to around 80° C., above which it remains the most prominent of the denaturable serum proteins until completely denatured with the heat treatment of 96° C. for 30 minutes. At 96° C., of course, only the ''proteose-peptone'' fraction remains and hence increases in relative concentration through the temperature range.

DISCUSSION

Smith *(19, 20)* reported the isolation of immune globulins from normal milk which are probably identical with or closely related to the immune globulins of blood serum. Although present at the highest concentration in colostrum, 8 to 20% of the serum proteins of normal milk were found to be composed of these globulins; this is apparently the material which has been identified as the classical ''globulin'' fraction of the milk serum proteins. Smith *(19)* further separated this fraction into a water soluble (euglobulin) and a water insoluble (pseudoglobulin) fraction and demonstrated mobilities of –1.5 to –1.7 and –2.1 to –2.5 for these components, respectively, under the same electrophoretic conditions used in this investigation. Components 1 and 2 in Figure 1 and Table 3 are thus identified as the euglobulin and pseudoglobulin components, respectively, of Smith. For calculation purposes in this investigation, both of these components were considered as the immune globulin fraction, and the patterns in Figure 1 indicate that they were heat-denatured at about the same rate.

The data in Table 1 indicate that the ''lactalbumin'' fraction quantitatively accounts for the proteins α-lactalbumin, β-lactoglobulin, and serum albumin. Since β-lactoglobulin composes 40 to 60% of the normal milk serum proteins, its denaturation curve closely resembles that of the complete serum proteins and also resembles the heat denaturation curve found for crystalline β-lactoglobulin measured by its loss in sulfhydryl group activity *(8)*. α-Lactalbumin is the most heat resistant of the denaturable serum proteins with over 50% of the original amount still remaining after the 77° C. heat treatment for 30 minutes, a heat treatment which denatured all of the immune globulins and the serum albumin.

Various investigators have attempted to characterize the proteins of the "proteose-peptone" fraction. Aschaffenburg *(1)* obtained 3% of the total protein of milk in a σ-proteose fraction which was prepared by salting out with one-half saturation of ammonium sulfate the proteins remaining in the serum after heating and removal of the casein and heat denaturable serum proteins at pH 4.5. The σ-proteose fraction was found to be very surface active. Weinstein *et al. (22)*, following essentially the procedure of Aschaffenburg except that the casein and heat denaturable serum proteins were removed with rennet, also isolated about 3% of the total proteins of milk in the form of a "minor protein fraction" which they believed to be associated with the solar activated flavor of milk. Electrophoretic analyses of this material were somewhat inconclusive, although more than one component was indicated. Since the "proteose-peptone" fraction of milk composes 2.8-5.3% of the total proteins *(17)*, it is apparent that the σ-proteose of Aschaffenburg *(1)* and the "minor protein fraction" of Weinstein *et al. (22)* contain a good part of the "proteose-peptone" fraction of milk.

The results of this investigation on the "proteose-peptone" fraction indicate the presence of a fairly well-defined fraction of electrophoretic homogeneity and two poorly defined fractions which are quite disperse in their electrophoretic properties. The major component ($\mu = -4.5$) has a mobility close to that of β-lactoglobulin ($\mu = -5.0$). From the patterns shown in Figure 2, it is apparent that this component was present in all of the solutions but was obscured by the large amount of β-lactoglobulin present in the low heated solutions. It is well to note that Smith *(19)*, using similar electrophoretic conditions, noticed a component in unheated milk serum with a mobility of –4.1 to –4.7 at a concentration of about 10%, which was observed only as an asymmetry on the side of the β-lactoglobulin peak. Stanley *et al. (21)* also observed a component with a mobility between that of α-lactalbumin and β-lactoglobulin (in a veronal–citrate buffer of pH 7.6) and which composed about 8% of the serum proteins. Gordon and Semmett *(4)*, using the electrophoretic conditions of Smith *(19)*, identified Component 5 ($\mu = -4.2$) as α-lactalbumin. Larson and Jenness *(9)* have shown, however, that Component 4 ($\mu = -3.7$) is the electrophoretic peak due to α-lactalbumin. In the present investigation, a concentration of 8.6% was found for Component 5 ($\mu = -4.5$) and this component can now be identified as a part of the "proteose-peptone" fraction of the milk serum proteins. With the identity of this fraction (Component 5) and also the part of the "proteose-peptone" fraction having a mobility greater than that of serum albumin (Component 8), apparently the major components evident at pH 8.6 in electrophoretic patterns of the normal unheated milk serum proteins have now been identified *(7, 12, 19, 21)*. It should not be assumed that the components of the three "proteose-peptone" fractions remain unchanged during the heating of the milk. The electrophoretic technique used in this study was not sensitive enough to detect or eliminate the possibility of the presence of small amounts of decomposition products of the original discrete proteins or the interaction products which one might expect to occur with a high heat treatment in the presence of lactose.

Hence, it might be expected that the "proteose-peptone" fraction isolated from a heated milk could be quite heterogenous while changing but little from the total amount present in the original milk. It is apparent that further work is needed to elucidate the material present in the "proteose-peptone" fraction.

The specific milk serum proteins have been shown to demonstrate a wide range of individual properties *(3, 4, 12, 15, 19)* and, in addition, they have now been shown to possess a difference in resistance to heat. Since the relative concentration of the individual proteins remaining undenatured in the serum changes with the amount of heat treatment given the original milk, within certain limits, an electrophoretic examination of the serum gives an indication of the history of previous heat treatment for a milk sample.

SUMMARY

Milk was heat-treated at eight temperatures between 56° and 96° C. for 30 minutes, and the proteins in the serum obtained after removal of the denatured serum proteins with the casein at pH 4.6 were examined by a quantitative electrophoretic procedure. The denaturation curves obtained for each of the milk serum proteins indicated that the immune globulins are the least, and α-lactalbumin the most, heat resistant, with β-lactoglobulin and serum albumin showing an intermediate sensitivity.

With the identification of three components in the electrophoretic patterns of the "proteose-peptone" fraction which were apparently present in the unheated milk, more of the electrophoretic entities of the milk serum proteins have now been elucidated.

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CHANGES IN BACTERIAL COUNTS AND FLAVOR OF DRY MILKS AFTER RECOMBINATION BY VARIOUS MEANS AND STORAGE AT LOW TEMPERATURES[1]

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Relatively large amounts of nonfat dry milk solids and dry whole milk are manufactured for use in recombined form for beverage purposes. Published information is lacking with respect to the keeping quality of such recombined products as affected by the growth of microorganisms.

The primary objective of the studies reported herein was to obtain information relative to the influence of certain recombination practices on the extent of bacterial growth and changes in flavor of recombined dried milks during storage at low temperatures. The factors studied were *(a)* effect of temperature of water used for recombination, *(b)* type of water used for recombination, and *(c)* consumer practices relative to recombination in the home.

MATERIALS AND METHODS

Products used in these studies were dry whole milk (DWM) representative of that made commercially by five manufacturers under contract with the Armed Forces and nonfat dry milk solids (NFDMS) manufactured for sale in retail consumer packages.

Special conditions of recombination are given in the descriptions of the various experiments presented below. It is emphasized that with the exceptions of the experiments involving recombination by consumers, all recombined products were prepared with the use of sterilized (autoclaved) equipment. Types of water used included sterile distilled water, tap water from the St. Paul city supply, and water from several rural and suburban private wells.

Recombined products were stored at temperatures ranging from 35° to 50° F.; the specific temperatures of storage were dependent upon the product used and the nature of the experiment. Each product was stored in a single container and in sufficient volume to allow rapid successive removal of samples for analysis; thus, the temperature of the products remained constant, for no detectable change in temperature occurred during the brief intervals of exposure at room temperature during sampling.

Bacterial counts and flavor characteristics were obtained initially and at subsequent intervals, usually 24, 48, and 72 hours.

After each storage period approximately 100 ml. of each product was removed

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for examination. A portion was used for bacteriological analysis and the remainder was used for flavor examination. In the experiments with NFDMS, flavors were described as good, fair, or poor, according to the method recommended by the American Dry Milk Institute *(1)*. Flavor scores for the recombined DWM were assigned in accordance with the U. S. Department of Agriculture Score Card for milk and cream.

Bacteriological examinations consisted of *(a)* standard plate counts, with incubation at 32° C., *(b)* psychrophilic counts, with incubation at 7° C. for 10 days, and *(c)* coliform counts, with incubation at 35° C. Tryptone glucose extract milk medium was used for *a* and *b* above. Violet red bile medium was used to obtain quantitative coliform counts. The presence of coliform bacteria in the waters was determined by inoculating 50 ml. of double strength lactose broth with 50 ml. of the respective water. Formation of gas within 24 hours incubation at 35° C., or formation of gas during an additional 24-hour incubation period with confirmation on eosin-methylene blue agar, was considered presumptive evidence of the presence of organisms of this group.

RESULTS

Effect of temperature of recombination on bacterial counts and flavor of recombined dry whole milk and nonfat dry milk solids during storage at 35° and 50° F.

Recombined dry whole milk. Four 1-gal. portions of recombined product were prepared from each of five lots of dry whole milk. One portion was prepared by recombination with sterile distilled water at 35° F., the second with sterile distilled water at 75° F., the third with tap water at 35° F., and the fourth with tap water at 75° F. Each of the four quantities was divided into two parts and one was stored at 35° F., the other at 50° F. In an attempt to accentuate the effect of high temperature of recombination, the portions prepared with water at 75° F. were held at room temperature for ½ hour before being placed in storage.

The procedure of recombination was as follows: 1 lb. of dry whole milk was added to 4 lb. of water in a metal mixing bowl. The mixture was agitated vigorously with a wire whip and made up to 8½ lb. with additional water. Erlenmeyer flasks with aluminum foil closures were used as containers for storage of the products.

A commercial milk dispensing cabinet and a 6 cu. ft. commercial refrigerator with forced air circulation maintained at 35° F. and 50° F., respectively, were used for storage. Since the temperature of water used in recombination may materially affect the length of time that the product may remain at temperatures more favorable for bacterial growth than the temperatures at which the refrigerators were maintained, cooling curves for the products recombined at 75° F. were obtained. This was done by recording temperatures at hourly intervals by means of thermocouples immersed in the products near the center of the containers. These curves are presented in Figure 1.

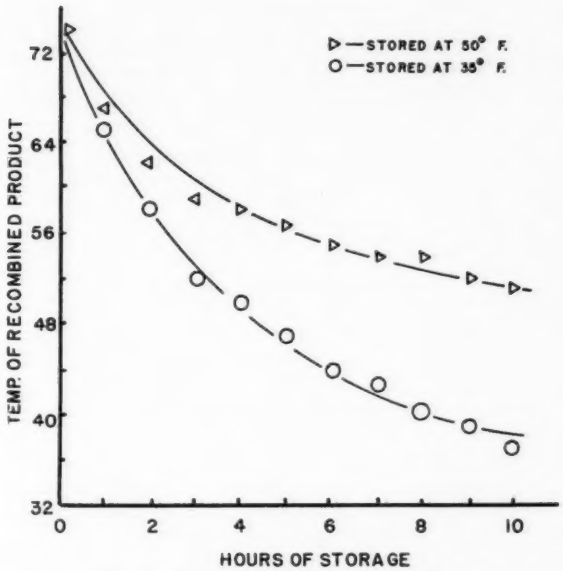

FIG. 1. Temperature changes during storage at 35° and 50° F. of dry whole milk recombined with water at 75° F.

The data shown in Table 1 are representative of those obtained from the five lots of DWM. In two of the trials the flavor scores decreased slightly after 24 and 48 hours. This decrease in score was due to the presence of a slight cabbage-like odor. It was the consensus of opinion that this odor was associated with a cooked flavor. In subsequent trials samples were agitated on a platform shaker for 10 minutes prior to examination for flavor. This eliminated the off odor, and flavor scores remained constant during storage. Bacterial counts remained at very low levels throughout the storage period. Although some increases in bacterial counts were observed in the products recombined at 75° F. and stored at 50° F., no evidence was obtained which would indicate that bacterial growth caused any adverse effect on the recombined milks.

Recombined nonfat dry milk solids, Trial 1. Four 1-qt. portions of recombined product were prepared from each of four lots of NFDMS. Two portions were recombined with tap water and two with sterile distilled water. The temperature of water used was 75° F. This experiment was intended, in part, to obtain information on the keeping quality of recombined milk prepared from several brands of NFDMS when recombined under conditions which would exclude contamination from external sources. The NFDMS were weighed directly into sterilized flasks containing the water, and agitation was done by means of a mechanical shaker. Immediately after preparation, one each of the products recombined with tap and sterile distilled water was placed in storage at 35° and 50° F.

TABLE 1

Bacterial counts and flavor of recombined DWM[a]

Water used	Temp. of water	Standard plate count	Psychrophile count	Coliform count
		(per ml.)	*(per ml.)*	*(per ml.)*
		Immediately after recombination		
Tap	75° F.	560	0	0
	35° F.	470	0	0
Sterile	75° F.	510	1	0
	35° F.	440	1	0
		After 24 hours storage at 35° F.		
Tap	75° F.	490	4	0
	35° F.	440	2	0
Sterile	75° F.	440	4	0
	35° F.	500	2	0
		After 48 hours storage at 35° F.		
Tap	75° F.	530	2	0
	35° F.	490	4	0
Sterile	75° F.	510	2	0
	35° F.	420	2	0
		After 24 hours storage at 50° F.		
Tap	75° F.	460	11	0
	35° F.	470	3	0
Sterile	75° F.	520	5	0
	35° F.	390	2	0
		After 48 hours storage at 50° F.		
Tap	75° F.	780	200	0
	35° F.	460	20	0
Sterile	75° F.	940	78	0
	35° F.	440	18	0

[a] All samples had cooked flavor and scored 36.

The data shown in Table 2 are representative of those obtained from the four lots of NFDMS. No change in flavor characteristics or grade occurred in any of the samples during the 72-hour storage period. None of the products showed any significant increase in standard plate count during the first 48 hours of storage. Increases were observed after 72 hours but the counts remained low. Initial psychrophile counts were negligible. These counts did not show any appreciable increases during the 72-hour period at 35° F.; however, in the samples stored at 50° F. the counts increased steadily and gradually approached the standard plate counts. Coliform counts of 13 of the 16 samples remained at zero per milliliter throughout the storage period. The detection of coliform organisms in three of the samples (at levels of 4, 42, and 98 per milliliter) after the third 24-hour storage period may be explained either by a very small number present initially but not detectable in 1-ml. amounts or by possible contamination during the experiment.

Trial 2. This trial was intended, in part, to obtain information on bacterial and flavor changes which might occur during storage of recombined milk pre-

TABLE 2

Bacterial counts and flavor during storage at 35° and 50° F. of NFDMS recombined with water at 75° F.[a]

Water used	Storage temperature	Standard plate count	Psychrophile count	Coliform count
		(per ml.)	*(per ml.)*	*(per ml.)*
		Immediately after recombination		
Tap	50° F.	760	1	0
	35° F.	760	1	0
Sterile	50° F.	590	2	0
	35° F.	590	2	0
		After 24 hours storage		
Tap	50° F.	550	10	0
	35° F.	460	7	0
Sterile	50° F.	580	3	0
	35° F.	610	3	0
		After 48 hours storage		
Tap	50° F.	650	44	0
	35° F.	460	2	0
Sterile	50° F.	480	60	0
	35° F.	550	0	0
		After 72 hours storage		
Tap	50° F.	2,400	650	0
	35° F.	610	1	0
Sterile	50° F.	1,700	770	98
	35° F.	540	1	0

[a] All samples had good but cooked flavor.

pared in relatively large volume and stored in a commercial bulk milk dispenser without benefit of cooling prior to storage. Two 5-gal. lots of recombined milk were prepared by using essentially the same procedures as were described above for dry whole milk. Tap water, at 75° F. for one lot and at 35° F. for the other, was used for recombination. Immediately after recombination both lots were placed in a bulk milk dispenser cabinet with the thermostat set to maintain a temperature of 35° F., as recommended by the manufacturer.

Temperature changes, as recorded by means of thermocouples placed near the top and bottom of each can, are shown in Figure 2. These curves were obtained by means of a separate trial which duplicated the conditions described above except that a temperature of 40° F. was used for recombination.

Samples for bacteriological and flavor examinations were drawn at the dispenser nozzles. The results as shown in Table 3 show slight increases in psychrophile counts during the 48-hour storage period but no changes in flavor. This is especially interesting in view of the fact that the temperature of the product recombined at 75° F. remained, at least in the top portion of the can, at temperatures above 50° F. for approximately 10 hours. It should be kept in mind, however, that since recombination was carried out in a manner to keep contamination at a minimum, only the original microflora of the NFDMS and of the water supply were present in the recombined product. With gross or even

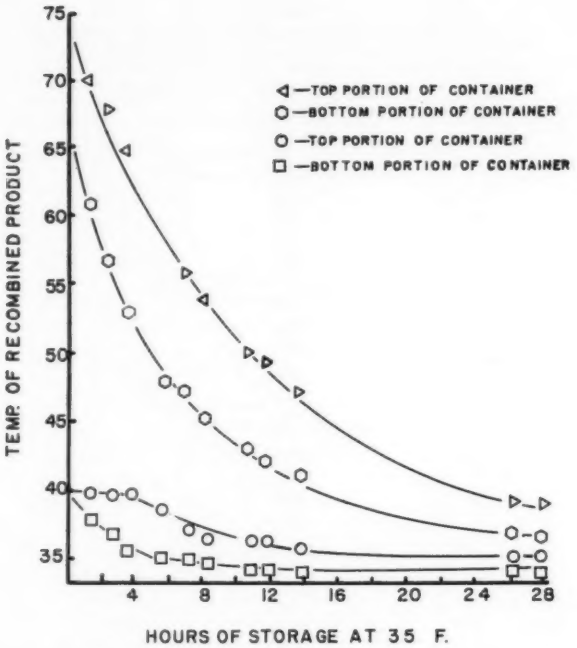

FIG. 2. Temperature changes during storage in a bulk milk dispenser of nonfat dry milk solids recombined with water at 75° and 40° F.

limited contamination, the situation with respect to flavor and microflora changes conceivably might have been different.

TABLE 3

Bacterial counts and flavor during storage in a bulk milk dispenser at 35° F. of NFDMS recombined with water at 35° and 75° F.[a]

Temperature of water used for recombination	Standard plate count	Psychrophile count	Coliform count
	(per ml.)	*(per ml.)*	*(per ml.)*
	Immediately after recombination		
75° F.	930	6	0
35° F.	580	4	0
	24 hours after recombination		
75° F.	630	3	0
35° F.	550	1	0
	48 hours after recombination		
75° F.	880	130	0
35° F.	610	110	0

[a] All samples had good but cooked flavor.

Changes in bacterial counts and flavor of NFDMS after recombination by consumers. The purpose of this experiment was to observe changes in flavor and bacterial counts during storage of NFDMS recombined and stored under circumstances representative of what might be expected under practical conditions in civilian and, to some extent, military kitchens, where this product may be prepared for beverage purposes.

One-pound consumer packages were distributed to eight homes where recombined NFMDS were used regularly. Consumers were asked to use the recombination procedure which in their own experience had given most satisfactory results. Immediately after recombination one quart of product and a sample of the water were brought to the laboratory. The recombined milks were stored at 50° F. The usual examinations were made after 24, 48, and 72 hours. The data shown in Table 4 are representative of those obtained from the consumer samples.

The flavor of every sample as received from participating consumers, includ-

TABLE 4

Bacterial counts and flavor of recombined NFDMS during storage at 50° F. after recombination by consumers[a]

Hours of storage	Standard plate count	Psychrophile count	Coliform count
	(per ml.)	*(per ml.)*	*(per ml.)*
	Consumer 8, trial 1		
0	6,000	570	0
24	6,000	3,200	0
48	11,000	6,600	0
72	740,000	560,000	0
water used	6,900	580	negative
	Consumer 3, trial 2		
0	1,500	380	0
24	34,000	26,000	0
48	7,200,000	7,400,000	0
72	42,000,000	40,000,000	0
water used	0	0	negative
	Consumer 4, trial 2		
0	50,000	490	350
24	63,000	45,000	600
48	770,000	410,000	—
72	23,000,000	26,000,000	31,000
water used	2	0	negative
	Consumer 6		
0	3,700	1,500	30
24	19,000	29,000	—
48	3,100,000	5,800,000	730
72	24,000,000	23,000,000	11,000
water used	1,800	270	positive
	Recombined in laboratory (control), trial 2		
0	530	2	0
24	930	2	0
48	820	33	0
72	850	500	0
water used	0	0	negative

[a] All samples had good flavor except 72-hr. sample prepared by Consumer 6, which was malty.

ing the controls prepared in the laboratory, was good. The flavor of the controls and 11 of the 13 consumer-prepared milks remained unchanged during the 72-hour storage period; one sample had a fermented flavor after 48 hours of storage and one a malty flavor after 72 hours.

The changes in bacterial counts were unlike any that had been observed in previous experiments. Bacterial counts of the 11 samples which showed no changes in flavor during the 72-hour storage period varied greatly; psychrophile counts of several million per milliliter after 72 hours of storage at 50° F. were not uncommon and in several instances equaled or exceeded the counts of samples which deteriorated in flavor. The importance of types of bacteria present rather than total population is emphasized by these results. Initially, five of the 13 consumer samples had coliform organisms present. Coliform counts of these samples increased slowly but never became great enough to account for any appreciable part of the total population as measured by the psychrophile count. In only one instance, however, (Consumer 6) was presumptive evidence of coliform contamination of a water supply obtained. This water was from a suburban well.

The bacterial counts of the well waters used for recombination probably were representative of what might be expected from an average group of suburban wells. This is borne out by counts from another series of wells as will be shown later. Standard plate counts of well waters ranged from 280 to 6,900 per milliliter, and psychrophile counts ranged from zero to 580 per milliliter. The lowest counts were obtained in water from the St. Paul city supply. In none of the seven such samples taken at homes of consumers was the standard plate or psychrophile count over two per milliliter.

The results of this experiment indicate strongly that contamination introduced from kitchen utensils during recombination was of much greater importance in accounting for excessive increases in bacterial counts and flavor changes than was the microflora of the water supplies or other sources, such as the hands or the air. Control samples, which were recombined in the laboratory with tap water and under aseptic conditions, showed only slight increases in bacterial counts during 72 hours of storage, whereas samples recombined by consumers showed significant increases in every instance. In fact, the highest count that was recorded initially was obtained from a sample prepared with water having a standard plate count of two per milliliter and a psychrophile count of zero per milliliter (Consumer 4, Trial 2). Likewise, one of the highest counts that was recorded after storage was obtained from a sample prepared with water showing no detectable organisms (Consumer 3, Trial 2). The water in both instances was from the St. Paul municipal supply. Furthermore, the effect of excluding contamination is evident when the initial counts of the control samples are compared with similar counts of other samples.

To supplement the data reported in Table 4, water was obtained from three additional suburban wells and used in recombination of NFDMS, the latter from the same source as that previously used. In this series, recombination was done in the laboratory under aseptic conditions. The results are shown in Table 5.

TABLE 5

Bacterial counts and flavor of NFDMS recombined with waters of varying bacterial quality and stored at 45° F. for 72 hours[a]

Hours of storage and type of water	Standard plate count	Psychrophile count	Coliform count
	(per ml.)	*(per ml.)*	*(per ml.)*
Sterile distilled	0	0	0
initial	60	0	–
24 hours	260	0	–
48 hours	310	0	–
72 hours	840	20	–
Well No. 1	20	10	0
initial	30	0	–
24 hours	80	30	–
48 hours	180	20	–
72 hours	760	370	–
Well No. 2	3,100	180	1
initial	2,500	630	–
24 hours	2,600	1,000	–
48 hours	20,000	9,500	–
72 hours	130,000	88,000	–
Well No. 3	60	100	0
initial	80	110	–
24 hours	180	—	–
48 hours	3,800	12,000	–
72 hours	450,000	1,200,000	–

[a] All samples had good flavor.

Bacterial counts increased somewhat during storage but did not reach levels of sufficient magnitude to affect flavor, or even to levels which would approximate those reached previously in several samples recombined with water containing few if any organisms (Table 4). These data when compared with those shown in Table 4 provide additional evidence with respect to the importance of kitchen utensil sanitation in contributing large numbers of bacteria to freshly recombined milk.

SUMMARY AND CONCLUSIONS

The effect of *(a)* temperature of recombination, *(b)* type of water, and *(c)* consumer practices on changes in bacterial counts and flavor of recombined dried milks was studied.

Water at a temperature of 75° F. when used for recombination had no effect on flavor of dry whole milk during subsequent storage at 35° or 50° F. for 48 hours. Bacterial counts were affected slightly, but the increases were too small to result in any adverse effect on the stored milks. Similar results were obtained in studies with nonfat dry milk solids (NFDMS).

The bacteriological quality of water supplies used by consumers in recombining NFDMS varied greatly. The bacteria contained in these supplies, although contributing significantly to the flora of freshly recombined milks, did not increase to populations of sufficient magnitude to affect adversely the flavor of the milks during storage at 45° or 50° F. for 72 hours.

NFDMS when recombined in the home generally deteriorated rapidly during storage at 50° F., as indicated by excessive increases in bacterial counts. Counts of several million per milliliter after 48 and 72 hours were not uncommon; however, in most instances the flavor of these milks remained unchanged. The type and temperature of water could not be shown to influence keeping quality under the conditions of these studies. The sanitary condition of kitchen utensils appeared to be the limiting factor in preparing recombined milk having satisfactory keeping quality.

REFERENCES

(1) American Dry Milk Institute, Inc. The Grading of Nonfat Dry Milk Solids and Sanitary and Quality Standards Including Standard Methods of Analysis. *Bull.* 911. 1948.

FURTHER STUDIES ON IN-PLACE CLEANING

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Many milk sanitarians and public health authorities are convinced that properly designed and properly installed sanitary pipe lines can be effectively cleaned in place. This work is a continuation of a study reported earlier *(5)* and was undertaken to obtain information on the effect on the sanitary condition of the line of *(a)* time of sanitization, *(b)* elimination of acid treatment, *(c)* reduction of circulation velocity, and *(d)* lowering the temperature of the cleaning solution. In addition, the effectiveness of in-place cleaning of an existing, conventional, take-down, sanitary stainless steel line, equipped with paper gaskets and Wizseals[1] was investigated.

EXPERIMENTAL PROCEDURE

A. PERMANENT LINE.

Description of line. The 1.5-in. stainless steel line used in this study was installed in the University of Illinois Creamery in 1950.[2] The line consists of 11 ft. 9 in. of 180-grit pipe; 10 ft. of 300-grit pipe; 20 ft. of 500-grit pipe; and 4 ft. 5 in. of electropolished pipe. Machined stainless steel clamp-type sanitary fittings with self-centering Teflon gaskets replaced the hexagonal union nut joint used by Moore *et al. (5)*. Details of this fitting and joint are shown in Figure 1.

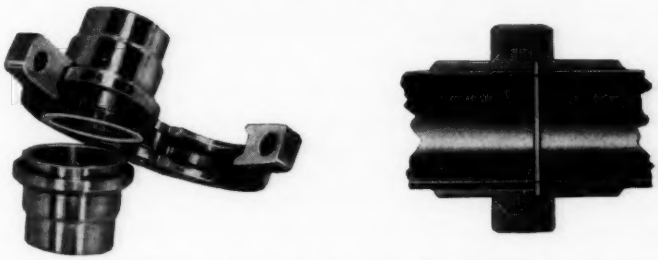

FIG. 1. Clamp-type fitting used in the permanent cleaned-in-place line.

The line carried hot products from the pasteurization vat to the cooler. An average of 2,000 lb. of whole milk was processed every other day. Skimmilk, ice cream mix, and chocolate drink also passed through the line. On days when the system was not regularly in use, skimmilk was recirculated at 150° F. for about 1 hour.

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[1] Wisner Manufacturing Corporation, 122 Hudson Street, New York, New York.

[2] The stainless steel tubing was supplied by the Carpenter Steel Company, Union, New Jersey, and installed and maintained by the Cherry-Burrell Corporation, Chicago.

Cleaning and sanitizing the line. Cleaning and sanitizing were accomplished by circulation, using a complete stainless steel system. The cleaning solution was made up in a 30-gal. stainless steel surge tank heated by steam injection and maintained at the desired temperature throughout the cleaning cycle by means of an automatically controlled valve. The standard cleaning procedure (Trial 1) was as follows:

(a) Flushing the line immediately after use with 30 gal. of tap water at 90° F. to remove residual traces of milk; this water was not recirculated.

(b) Recirculating 0.75 pt. of liquid acid cleaner in 30 gal. of water at 160° F. for 15 minutes at a velocity of 13.4 ft. per second and flushing with 30 gal. of water at 90° F.

(c) Recirculating 30 gal. of an alkaline cleaning solution at 160° F. containing the minimum recommended level of 0.75 lb. of cleaner[3] for 15 minutes at a velocity of 13.4 ft. per second.

(d) Rinsing with water as in *a*.

(e) Sanitizing immediately after cleaning with 30 gal. of water containing 200 p.p.m. of available chlorine. This required 5 minutes.

(f) Sanitizing before use as in *e*.

The above procedure was patterned after that suggested by the 3A Sanitary Standards Committee *(2)*. It was modified by eliminating certain operations to determine the importance of each on the sanitary condition of the line.

Sanitization immediately after clean-up was initially incorporated as a safety measure; the role of this particular operation on the final sanitary condition of the line was investigated in Trial II. The significance of the acid treatment was determined in Trial III, and the importance of the velocity of the circulating cleaning solution was studied in Trial IV. In view of the fact that the suggested cleaning procedure of the 3A Standard Committee *(2)* recommends the use of a nondepositing alkaline solution at 120° F., or above, the efficiency of the alkali at this temperature was investigated in Trial V.

Examination of the line. A bacteriological examination and visual inspection were made at the end of each experimental cleaning period (90 days for Trial I and 14 days for the other trials). The bacteriological swab technique, as described by Moore *et al. (5)*, was used to determine the sanitary condition of the line. Swabs were made from the face of the ferrule proper and from a 4 sq. in. area of the interior of the pipe. All swabs were made after chlorine sanitization. They were immediately placed in 4 ml. of buffer solution containing thiosulphate. One milliliter of this solution was plated. The results were reported as the number of bacteria per 4 ml., which represents the total number of contaminating organisms on the area under examination.

Because of the importance of even small numbers of psychrophiles in pasteurized milk and cream *(3, 6, 7)* studies were also made to determine the extent of this contamination in pipe lines. All bacteriological platings were carried out with standard procedures. When making the psychrophilic counts, the plates

[3] The cleaning compound consisted of polyphosphates, metasilicate, soda ash, and nonionic wetting agent.

were incubated at 10° C. for 7 days. Coliform determinations were made on violet red bile agar. The degree of cleanliness was also ascertained by visual inspection.

B. TAKE-DOWN LINE.

Description of line. This line, which had been in use for several years for cold milk products, consisted of 55 ft. of 1.5-in., 180-grit, 18-8 stainless steel pipe and contained 15 unions all secured by means of standard hexagonal nuts. Twelve joints included standard bevel seat ferrules; two consisted of a combination of a bevel seat ferrule and a flat-face gasket seat ferrule, and one consisted of two

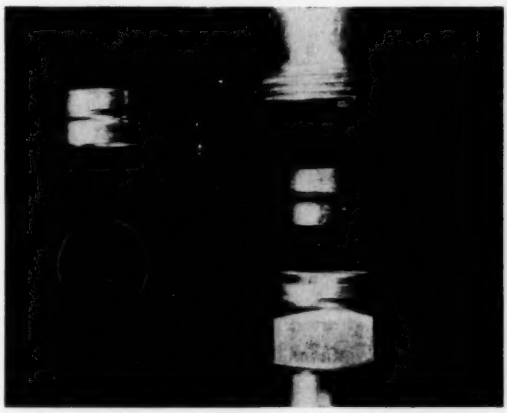

FIG. 2. Wiz-seal used in place of paper gaskets for in-place cleaning of a conventional take-down line.

bevel seat fittings that had been converted to gasket seat fittings. Three types of gaskets were used in this line over a 9-day period. In the first studies, paper gaskets were used in ten of the bevel seat unions, and Wiz-seals (Fig. 2) were used in the remaining two joints of this type. Teflon gaskets were used at the other joints. After 9 days the paper and Teflon gaskets were all replaced with Wiz-seals, and the study continued for 1 year.

Cleaning and sanitizing the line. When comparing different gaskets, recirculation cleaning was undertaken by using an alkaline solution at 120° F., as described in Trial V (Table 1). The line was operated for a 3-day period and a 6-day period under these conditions. After the paper and Teflon gaskets were replaced with Wiz-seals, the line was cleaned by flushing with warm (90° F.) water and by recirculating for 15 minutes a solution of alkali cleaning powder (1.25 lb. per 30 gal.) at a temperature of 145°-150° F. The cleaning solution was flushed from the line with warm water; no acid treatment was used. A chlorine solution (200 p.p.m.) was pumped through the line 1 to 2 hours before use.

Examination of the line. After being in operation 6 days, the unions and line

TABLE 1

Sanitary condition of permanent CIP line showing average mesophile counts after various cleaning procedures

Location swabbed	Cleaning procedure									
	Trial I[d] Acid Alkali Sanitize[a] Vel. 13.4 T. 160° F.		Trial II[d] Acid Alkali ——— Vel. 13.4 T. 160° F.		Trial III[d] ——— Alkali ——— Vel. 13.4 T. 160° F.		Trial IV[d] ——— Alkali ——— Vel. 4.8 T. 160° F.		Trial V[d] ——— Alkali ——— Vel. 4.8 T. 120° F.	
	(Av. No.)[b]	*(% Neg.)*[c]	*(Av. No.)*	*(% Neg.)*	*(Av. No.)*	*(% Neg.)*	*(Av. No.)*	*(% Neg.)*	*(Av. No.)*	*(% Neg.)*
Face of joint	32	10	12	25	12	35	6	35	6	35
4 sq. in. of pipe	16	38	4	37	8	50	4	61	2	60
Visual inspect.	Clean No film		Clean No film		Clean No film		Clean No film		Rainbow discoloration	

[a] Sanitization immediately after clean-up; omitted in other trials.

[b] Figures represent the average mesophile count per area examined; psychrophiles were found only in Trial I; no coliforms were observed in any trials.

[c] Per cent of samples showing no growth of mesophiles.

[d] Duration of trial 14 days, except Trial I, which was run for 90 days.

were examined bacteriologically and visually. The condition of the paper gaskets was such that they were replaced. On the ninth day, the line was again examined and because of the poor condition of the paper gaskets they were all replaced with Wiz-seals. After being in operation for 1 year, the line was taken apart and thoroughly checked for bacterial contamination and apparent cleanliness.

RESULTS AND DISCUSSION

A. PERMANENT LINE.

The results of the in-place-cleaning studies made on the permanent line are summarized in Table 1. In Trial I, the mesophile count from eight joints ranged from 0 to 256 (av. 32). Counts of less than one per area swabbed were obtained in 10% of the tests. The psychrophile counts on the joints varied from 0 to 48 (av. 12); 50% of the areas examined showed no growth. No coliform contamination was observed in the joints.

The mesophile counts on five sections of pipe in Trial I ranged from 0 to 104 (av. 16) per area swabbed; 38% of the areas showed no growth. The counts obtained from the pipes with different finishes were averaged together, since Moore *et al. (5)* showed no significant effect of grit finish on the sanitary condition of the pipe. An average psychrophile count of 5 per pipe area was obtained. A visual inspection of the joints and pipes at the conclusion of the experimental cleaning period showed the line to be clean and free of film.

In Trial II sanitization immediately after cleaning was eliminated. This procedure was originally incorporated to lower the residual bacterial contamination by reducing growth and multiplication during the period in which the line was not in use. Mesophile counts at the joints ranged from 0 to 40 (av. 12); 25% of the areas showed no growth. The mesophile count on the pipes ranged from 0 to 24 (av. 4), with 37% of the areas showing less than one organism per area examined. An inspection of the line at the end of the test period showed it to be clean and bright in appearance. The bacteriological findings and physical appearance of the line compared favorably with those obtained in Trial I. From this it can be concluded that chlorination immediately after cleaning does not sufficiently improve the final sanitary condition of the line to justify the expense of the additional operation.

Eliminating the acid cleaning procedure in Trial III did not affect the sanitary condition of the joints or pipes. The mesophile count on the former ranged from 0 to 48 (av. 12) with 35% of the areas showing no evidence of bacterial contamination. The mesophile counts on the pipe areas ranged from 0 to 56 (av. 8) per area swabbed; 50% of the areas showed no growth. Visual examination showed no evidence of film formation or discoloration.

Reducing the flow velocity of the alkali cleaning solution in Trial IV did not adversely affect sanitization of the line. Mesophile swab counts ranged from 0 to 16 (av. 6) per joint, with 35% of the areas showing no growth. The mesophile count of the pipes also ranged from 0 to 16 (av. 4) and 61% of the areas examined showed no contamination. No staining or film formation was noted. From

this it was concluded that, under the conditions of this experiment, successful cleaning can be accomplished by flushing the line with water and circulating this alkaline cleaning solution at a temperature of 160° F. and a velocity of 4.8 ft. per second.

In Trial V the temperature of the alkaline cleaning solution was reduced from 160° to 120° F. From a bacteriological point of view the joints and pipes remained in an excellent state of sanitation, as evidenced by the mesophile counts, which ranged from 0 to 8 (av. 6) in the former and from 0 to 8 (av. 2) in the latter. Excellent bacteriological results are indicated by the fact that no contamination was observed in 35% of the joints and in 60% of the pipes.

A visual examination of the line, however, showed stained areas which presented a typical iridescent sheen similar to that obtained with an oil film on water. At the end of the two-week period, the discolored areas appeared as blotches. The period of the trial was extended for four more weeks. At the end of this time, the rainbow discoloration extended throughout the entire length of all the pipes. It has been observed by the authors that a discoloration of this type exists rather commonly on commercial stainless steel pasteurizing equipment.

An attempt was made to study this phenomenon. At this point in the study, the supply of nonionic cleaner became exhausted and it was necessary to change to an anionic cleaning powder. Using the latter at 120° F. failed to remove the film; raising the temperature to 160° F. removed the film. An effort was made to reproduce the film with anionic cleaning solution at a temperature of 120° F., but even after 1 year no iridescent sheen was observed. This phase of the study is under further investigation.

A sample of the discolored stainless steel was subjected to electron diffraction studies. The results showed that the colored areas were metal oxides and not organic or inorganic deposits. Recently Lewandowski *(4)* reported the appearance of an iridescent discoloration on electroplated tinned steel strips after exposure to acetic acid. Stainless steel (18-8), however, showed no discoloration under the same conditions.

Although the bacteriological findings after a 2-week test period on the permanent line indicated a satisfactory sanitary condition, swab counts made after operating for 1 year with the cleaning schedule described in Trial V showed that some bacterial build-up occurred. At three joints the counts were less than 30; in the remaining five the counts ranged from 1,000 to 32,000. Improper line maintenance accounted for three high counts. It is noteworthy that in one of the latter, the original ferrule was removed from the pipe and rewelded in the plant shop; rewelding misshaped the ferrule to the extent that it was necessary to hammer the joint to separate it. This poor-fitting joint also caused the line to be misaligned, and leakage occurred at two elbows, as indicated by an accumulation of milk solids on the exterior of the pipe. It is apparent that improper engineering in installation or improper maintenance of a cleaned-in-place line may make it impossible to achieve the degree of sanitation which normally would be obtained with such a system.

B. TAKE-DOWN LINE.

The bacteriological results obtained after in-place cleaning of a standard take-down line are shown in Table 2. Operating the line for only 3 days resulted in an enormous bacterial build-up at joints with paper gaskets. It is apparent from this that paper gaskets cannot be cleaned or sanitized by circulation cleaning. After a 6-day test period, the counts obtained at unions consisting of a combination of a standard bevel ferrule and a flat-face fitting or two flat-face fittings with Teflon gaskets compared favorably with those obtained with special permanent-type fittings and clamps.

TABLE 2

Sanitary evaluation of conventional line fitted with various gaskets and cleaned in place[a]

Description of joint examined	No. of joints examined	Type of gasket	Mesophiles after operating		Visual observation
			6 days	3 days	
Two bevel fittings	10	Paper	TNTC[b]	TNTC	Sl. odor Gaskets soggy
Bevel fitting and flat fitting	2	Teflon	0-4	0	No odor Unions clean
Two flat-face fittings	1	Teflon	0-4	0	No odor Unions clean
Two bevel fittings	2	Wiz-seals	0-8	0-4	No odor Unions clean

[a] Cleaned under conditions described in Trial V.

[b] TNTC indicates a count greater than 8,000; figures represent number of colonies per area examined.

Conventional bevel fittings containing standard Wiz-seals gave low swab counts after the 6-day experimental period. After in-place cleaning for 1 year, two of the 15 joints containing Wiz-seals showed no evidence of bacterial contamination; two joints showed counts of less than 10; six joints showed counts of less than 100; two joints showed counts of less than 1,000, and three joints showed counts between 4,000 and 20,000. In one of these three joints, the Wiz-seal was very badly split as the result of excessive tightening. The two other high counts were obtained at joints with valves. In both cases a rust spot about 0.5 sq. in. in area was observed on the body of the valve into which the plug fits. It was observed that one of the seals fit loosely into the valve body and the other tightly. In the former the rust spot occurred under the seal; in the latter it was adjacent to the seal.

It is interesting to note that no rusting occurred at other joints. It is possible that removing the valve plug to hand-brush the body of the valve may have permitted cleaning compound to accumulate behind the shoulder or under the rim and provided favorable conditions for corrosion.

The minimum recommended pitch generally used for a cleaned-in-place line is 1 in. per 8 ft. *(1)*. The take-down line in this study had a pitch of only 0.5 in. per 8 ft. When the line was disassembled for cleaning, this was sufficient as each

pipe was drained before assembling. When a cleaned-in-place procedure was used, however, the final rinse water collected behind the dam formed by the Wiz-seal and caused a greyish film of hard water deposit to form on two sections of pipe. One can conclude from this that some consideration must be given to pitch when these gaskets are used to replace paper gaskets in a conventional take-down line. The pitch was decreased still further when the take-down line was connected to the existing cleaned-in-place facilities for chlorination as it was necessary to raise the insecurely anchored line to obtain proper alignment. This allowed sanitizing solution to collect in the line, and corrosion occurred in 15 ft. of pipe. Insecure anchoring of the line was responsible for the damaged rubber ring of the Wiz-seal, as the pipe twisted in tightening and cut the rubber. In this regard, it is important to point out that lines with Wiz-seals should not be broken into or taken down frequently, since this causes undue twisting and straining at the joints and may cut gaskets. One of the aims of this study was to determine whether or not it was possible to substitute Wiz-seals for paper gaskets in a take-down line and to clean the existing line by in-place-cleaning procedures. It is possible to obtain satisfactory cleaning when Wiz-seals are used, but due regard must be given other factors such as pitch and proper hangers, which also affect the efficiency of the cleaning operation.

SUMMARY AND CONCLUSIONS

Chlorination immediately following the cleaning operation did not improve the final sanitary condition of the cleaned-in-place line sufficiently to warrant the additional expense.

Clean pipes were obtained in a permanent line used for hot milk products without the use of acid; no milkstone build-up was observed.

Circulation cleaning in a permanently installed 1.5-in. line at a velocity of 4.8 ft. per second was as efficient as circulation at 13.4 ft. per second.

Circulation cleaning with a nonionic cleaner at 120° F. produced a rainbow discoloration on the interior of the pipes. Circulating an anionic type cleaning compound at 120° F. failed to remove the film; at 160° F. the discoloration disappeared.

No coliform contamination was observed in the permanent system after sanitization. An extremely low level of psychrophilic contamination was observed in one trial; in four trials no organisms of this type were found.

Improper maintenance of permanent-type fittings resulted in an unclean and insanitary line; proper installation, as well as proper care after installation, is necessary to maintain permanently installed lines in satisfactory condition.

Paper gaskets in a conventional take-down line carrying cold milk could not be cleaned by means of circulation cleaning.

Wiz-seals in standard bevel joints may be cleaned in place under certain conditions; proper pitch and line installation are necessary for maximum cleaning efficiency.

ACKNOWLEDGMENTS

The authors wish to thank G. L. Clark, of the Chemistry Department, for the electron diffraction studies made on the iridescent film. The assistance of V. L. Swearingen in carrying out these studies is greatly appreciated. Thanks are due also to Z. J. Ordal for his suggestions in the initial phase of this work.

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INFLUENCE OF SUBLETHAL TREATMENT WITH HEAT OR CHLORINE ON THE GROWTH OF PSYCHROPHILIC BACTERIA[1]

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Bacteria which grow at low temperatures are of extreme importance to the dairy industry. Their presence in pasteurized milk apparently is due largely to post-pasteurization contamination, as several workers *(3, 8, 10, 11, 12)* have reported that psychrophilic bacteria do not withstand pasteurization.

The use of heat, chlorine, or quaternary ammonium compounds for sanitizing dairy equipment prior to use for handling pasteurized products is a common practice in the dairy industry. The time and temperature of exposure during these treatments may be difficult to control under certain conditions, permitting survival of some organisms. Preliminary work showed that cultures exposed to sublethal heat treatments sometimes reached high population levels at different rates than did cultures not subjected to heat. The magnitude of this difference depended somewhat on the temperature at which the culture was held for growth subsequent to heat treatment. Nelson *(6)* has reported that heat-treated cells may have more exacting requirements for growth; thus, the medium used in studying survival may be a factor influencing the results of studies on survival. Because of the possible practical significance of the preliminary findings, more detailed studies were carried out. The work also was extended to see if a similar effect resulted from partial inactivation by chlorine.

EXPERIMENTAL METHODS

Methods of isolation and handling pure cultures were outlined in a previous paper *(4)*. The cultures were grown in skimmilk for 10 days at 5° C. prior to heat treatment or use as a control. Detailed studies showed that the results were essentially the same whether the cultures were grown at 5°, 10°, or 25° C. as long as the cells were in the same growth phase when used. The partial inactivation by heat was carried out on 1.5 ml. of culture sealed in a 10 × 75 mm. soft glass test tube. The tubes were completely immersed in a water bath, heated, subsequently cooled in ice water, and opened as soon as possible, and 1 ml. of culture was transferred to 99 ml. of sterile skimmilk, the entire procedure requiring approximately 15 minutes. This preparation was plated at zero time and divided into three screw-cap test tubes to be held at 5°, 10°, and 25° C. When required to reduce the population to countable levels, the survivors were further diluted. The control was inoculated at the same time as the heated sample and

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held at room temperature for the approximately 15-minute interval preceding zero time. Studies showed that holding for as long as 3 hours under these conditions gave only a barely detectable and transitory greater increase in count early in the logarithmic phase of growth, so the 15-minute interval could be considered as of no significance. Platings were made every 24 hours on Tryptone Glucose Extract (TGE) agar *(1)* or Tryptone Glucose Yeast Extract (Plate Count) agar *(2)*. Plate incubation was for 3 days at 25° C., as suggested by Nelson and Baker *(7)*. Preliminary studies showed that this time and temperature gave results similar to those obtained by incubation for 7 days at 5° C.

During each trial a control was run by diluting an unheated culture so the initial concentration was in the same range as the expected survival of the heat-treated culture.

Partial inactivation by chlorine was accomplished by adding 1 ml. of a 1:100 dilution of a culture to 10 ml. of the appropriate strength chlorine solution. The mixture was shaken and allowed to stand for 1 minute; 1 ml. was then transferred to 99 ml. of sterile skimmilk and plated at zero time. The milk was divided into three screw-cap test tubes for holding at 5°, 10°, and 25° C. To check the count of the untreated culture, a control was run with sterile distilled water in place of the chlorine solution. Sampling and plating were done in the same manner as for heat-treated culture. Strength of chlorine solution was determined by the thiosulfate method *(5)*. The resulting amount of available chlorine was divided by two, as one atom of chlorine is equivalent to two molecules of thiosulfate *(9)*.

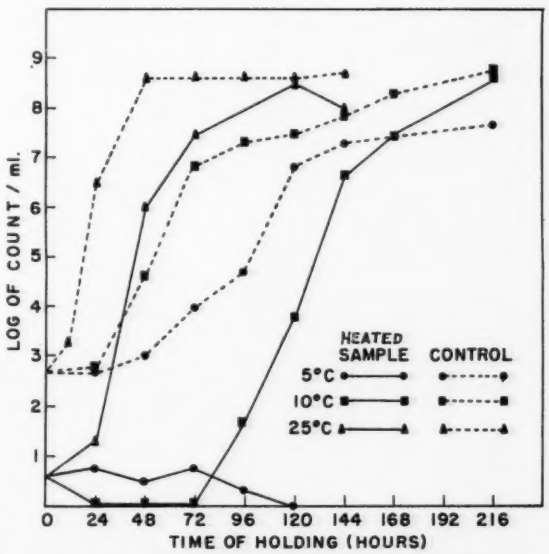

FIG. 1. Effect of partial destruction by heat on growth of *Ps. fluorescens* in milk held at three temperatures. Counts on TGE agar. (Initial count 46×10^7/ml.)

RESULTS

Pseudomonas fluorescens, Pseudomonas sp., *Ps. ovalis,* and *Ps. geniculata,* which had been isolated from milk *(4),* were studied during the experiment. Figure 1 shows the growth curves of a culture of *Ps. fluorescens* after heating for 7 minutes at 52° C. The count was 46×10^7 per milliliter prior to heating and the survivor count was 13×10^2 per milliliter, or about 0.0003% of the initial count. The count of the treated sample held at 5° C. remained at less than 10 per milliliter for the first 72 hours and then decreased to an undetectable level, with no reappearance by the end of 216 hours. Holding at 10° C. resulted in the treated culture remaining at or near zero count for 72 hours, followed by a fairly rapid rise to the same level as the control after 216 hours. When holding of treated sample was at 25° C., the shape of the curve seemed to be unaffected by the heat treatment, except for an increase of about 12 hours in the lag phase. Once growth had begun, the surviving population increased at a rate nearly parallel to that of the control at each temperature.

Figure 2 shows the results obtained when *Pseudomonas* sp. was heated for 9 minutes at 52° C. The initial count was 14×10^7 per milliliter, and the survival level was 0.043%. The data indicate that holding at 5° C. permitted no increase in count until 144 hours, whereas holding at 10° C. resulted in a drop in numbers followed by a normal rate of increase after 48 hours. Again, holding at 25° C. resulted in growth of survivors that was very similar to the control, except for evidence of a slightly prolonged lag phase.

Further studies with these cultures and also with *Ps. ovalis* and *Ps. geniculata*

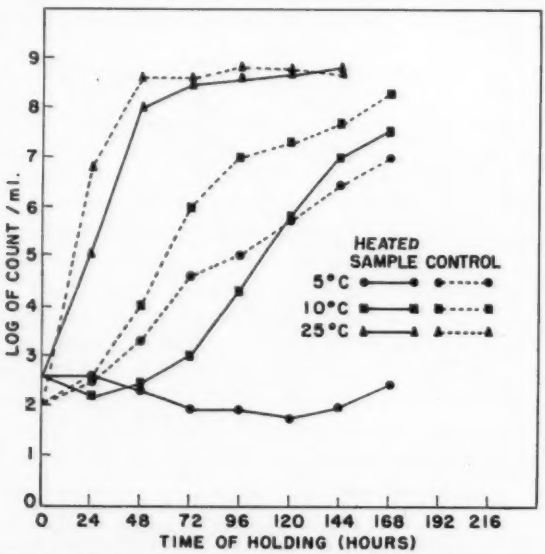

FIG. 2. Effect of partial destruction by heat on growth of *Pseudomonas* sp. in milk held at three temperatures. Counts on TGE agar. (Initial count 14×10^7/ml.)

gave similar results. In some cases the lag phase obtained at 5° C. was not so pronounced as the illustrations used, but it was seldom less than 72 hours.

When survivors of heat treatment were further diluted before holding, the resulting growth curves were nearly always parallel to the original survivor curves and separated from them only by the difference in initial inoculum. Thus, the absolute level of survivor population did not seem to be a major factor in the shape of the growth curve.

During the course of this work Plate Count agar was adopted as one of the new standard plate count media *(2)*, and it was thought advisable to compare it with TGE agar for the enumeration of heat-treated cultures. Duplicate sets of plates were poured from a single set of samples and dilutions, one set for each type of agar. The counts of *Ps. fluorescens* obtained on the two agars are plotted in Figure 3. Heating was at 52° C. for 5.5 minutes, instead of for 7 minutes as in Figure 1. The unheated controls showed similar curves on both types of agar; however, the count measured on TGE agar was considerably less than that obtained on the new Plate Count agar. The counts on samples held at 5° C. and plated on TGE agar rose slightly and remained stable until 95 hours, whereas the count on the Plate Count agar, which at zero time was considerably higher, dropped until it was similar to that on TGE agar after 48 hours and remained nearly equal throughout the balance of the test period. When storage was at 10° C. the counts on the two agars were essentially the same after 24 hours and remained nearly identical for the remainder of the observation period. After storage at 25° C., the counts on the new medium indicated a 24-hour lag in growth, followed by rapid growth; on TGE agar no such lag was indicated. The

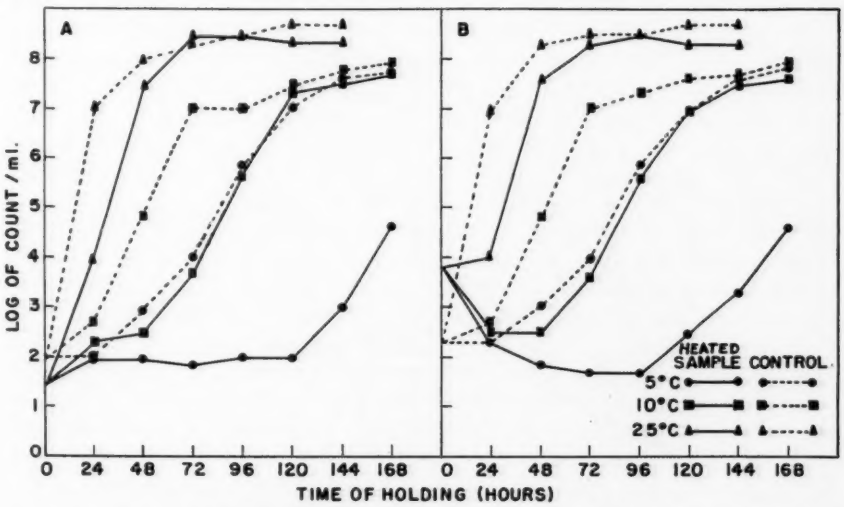

FIG. 3. Influence of plating medium on counts of *Ps. fluorescens* subjected to partial destruction by heat. A—plating on TGE agar. B—plating on Plate Count agar. (Preheating count 17×10^7/ml.)

counts on the two agars were almost exactly the same at 24 hours and beyond. The lag observed on Plate Count agar at 25° C. could be due to enumeration, at zero time, of many organisms which were not enumerated on TGE agar and which were unable to grow in milk. The organisms able to grow were possibly about the same as those enumerated on TGE, with the excess dying off during the first 24 hours. The net gain in viable organisms would not be great, resulting in an apparent lag phase. When TGE agar was used these extra organisms were not enumerated so there was no die-off noted to give a lag phase. Similar results were obtained with two other cultures.

The results obtained with heat-treated cultures prompted a similar study with cultures which had been partially inactivated by chlorine. Figure 4 shows the results of submitting a culture of *Ps. fluorescens* to an initial concentration of 10 p.p.m. of chlorine for 1 minute, followed by inoculation into milk and holding at 5°, 10°, and 25° C. The initial count, due to the dilution of 1:100 required in the procedure, was only 28×10^5 per milliliter. The figure indicates that the rates of increase of the treated samples were about the same as those of the controls at all storage temperatures. When chlorine-treated cultures were plated on TGE agar the counts were similar to parallel counts on Plate Count agar. Obviously the effect of chlorine on the cultures is different from that of heat.

DISCUSSION

The results obtained in this work indicated that the organisms which survived heat treatment required somewhat different conditions for resumption of normal growth than did the same organisms not exposed to heat. The difference was

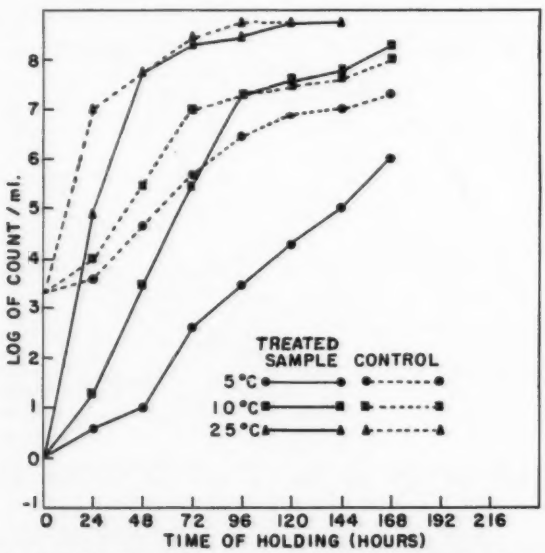

Fig. 4. Effect of partial destruction by chlorine on growth of *Ps. fluorescens* in milk at three different temperatures. Counts on TGE agar. (Initial count 28×10^5/ml.)

evident primarily in the lag phase of the growth curve, with the difference depending on the temperature of holding during resumption of growth. In some cases in which survival was low and milk was held at 5° C., the plate count on the culture continued to decline on holding until it was no longer possible to enumerate viable cells. This result could be explained by a low level of survival with a die-off, in the early period, great enough to eliminate organisms viable under the enumeration conditions employed. At 10° C. there was recovery after a prolonged lag phase, but at 25° C. only a slightly increased lag phase was noted, which could be explained by a less severe die-off or more favorable conditions for initiation of growth so recovery took place before all viable cells were lost. Some doubt was cast on this explanation when it was shown that smaller numbers of survivors recovered nearly as readily as larger numbers.

In some cases recovery occurred at 5° C. after a prolonged lag phase, and in others the surviving population remained fairly stable or declined to an uncountable level from which there was no recovery. These differences may be explained by a slight, uncontrollable variation of heat treatment. When damage was greater, the adjustment necessary for resumed growth was so great that it could not be made before die-off eliminated all viable cells. When damage was less severe, recovery was able to take place before all viable cells were eliminated, or at least the increase in numbers was able to balance the die-off.

Duplicate runs using TGE and Plate Count agars showed a consistent difference. The higher plate counts, obtained in the early part of the growth curve, on the Plate Count agar may have been due to conditions in the agar being more satisfactory for enumeration of heat-injured cells, than when TGE agar was used. Larger numbers were enumerated on Plate Count agar, but there was possibly no chance for these organisms to survive in milk and they probably were lost during the first 24-48 hours storage. Hence, subsequent platings yielded similar counts on both agars and tended to indicate a large drop in numbers when Plate Count agar was used. Another possible explanation could be that temporary inactivation of some enzyme system in some of the bacteria may have made it necessary to have a substrate supplied, which was present in Plate Count agar but not in TGE agar or in milk. The organisms requiring the substrate would quickly die out when held in milk. Thus, enumeration at 24, 48, and subsequent hours showed a similar count on both agars. This type of heat effect could be operating at the same time as the previously discussed phenomenon of die-off and recovery. Here again, some of the effect could be masked by the more rapid growth at 10° and 25° C.

Organisms surviving chlorine treatment were able to grow, at all temperatures tested, at a rate very similar to that of the untreated controls. The results indicated that treatment with chlorine resulted in an all-or-nothing effect, in contrast to heat, which was able to modify the growth of the survivors.

The results obtained on the influence of sublethal heat upon prolongation of lag phase of pure cultures may partially explain the commonly observed slow rate of initiation of growth of bacteria in some lots of refrigerated product. Previous studies *(4)* have shown that low temperatures alone do not prolong

unduly the lag phase of cultures not subjected to adverse conditions. In the present work the only adverse conditions studied have been temperature and chlorine treatments. In dairy plant operations residual bacteria might be subjected to desiccation, strong alkalies, radiations, and other factors that were not included in these investigations but might have a definite influence on initiation of growth. Certainly inadequate heat treatment seems to be a factor of possibly considerable significance in determining that some samples will show a prolonged lag phase for the bacteria growing at refrigeration temperature.

CONCLUSIONS

1. When a culture was partially killed by heat, the survivors had a greatly increased lag phase when holding was at 5° or 10° C. A slightly increased lag phase was noted when holding was at 25° C.
2. Immediately after heating, larger numbers of survivors of sublethal doses of heat were enumerated on Plate Count agar than on TGE agar, but by 24 hours this difference had largely disappeared.
3. The growth curves of organisms surviving treatment with chlorine were not altered to any great extent.

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REICHERT-MEISSL NUMBERS OF BUTTERFAT FROM COMMERCIAL PRODUCTS PRODUCED IN KANSAS[1]

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The Reichert-Meissl number of butterfat has been used extensively as an index of butterfat adulteration. One disadvantage of using this method to detect butterfat adulteration is the inherent variation in glyceride composition of butterfat, which results in variations in Reichert-Meissl numbers. There is apparently some doubt as to the normal variation of Reichert-Meissl numbers of butterfat. An arbitrary standard of approximately 24 to 33 has been accepted for many years in the United States and abroad *(3, 9, 16)*, although evidence in the literature indicates that butterfat from individual cows may have a Reichert-Meissl number as low as 14 *(8, 15)* under extreme feeding or environmental conditions. The generally accepted range of Reichert-Meissl numbers of butterfat is purely an arbitrary standard and should, therefore, be evaluated under present conditions of milk production. This paper reports the results of surveys designed to establish the normal range of Reichert-Meissl numbers of butterfat as found in commercial dairy products in Kansas.

EXPERIMENTAL PROCEDURE

The investigation was divided into two phases. In phase one a survey was made to determine the range of Reichert-Meissl numbers of butterfat as found in commercial ice cream during August. This time of year was selected because it has been reported that Reichert-Meissl numbers of butterfat are generally lower at that time than at any other period *(5, 6)*. The second phase of the investigation was a 1-year study of Reichert-Meissl numbers of commercial butter produced in 10 butter-producing plants in Kansas.

During the middle of August, 169 pint samples of ice cream were collected from many sections of Kansas. These samples, which included all of the flavors normally found in any market, were representative of practically all of the ice cream manufactured in the state and were believed to contain representative late summer butterfat. The samples, which were collected over a 5-day period, were stored at −8° C. until they were analyzed. Butteroil was prepared from ice cream samples by a demulsification and churning procedure in which Minnesota Babcock reagent was used as a demulsifying agent *(11)*. Samples were analyzed in duplicate for their Reichert-Meissl numbers, according to the procedure of the Association of Official Agricultural Chemists *(2)*. Number 2 Whatman filter paper was used to separate the water insoluble acids from the

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soluble acids after the saponified fat had been distilled and the distillate had been tempered.

During the 1-year period 105 samples of commercial butter were collected from 10 of the largest butter-producing plants in Kansas. Samples were taken at each plant at approximately the 15th day of each month. With few exceptions, butter was sampled from the same plants each month. Butteroil was prepared and Reichert-Meissl numbers were determined in duplicate, according to the official methods of the Association of Official Agricultural Chemists *(2)*.

RESULTS AND DISCUSSION

The Reichert-Meissl numbers of late summer butterfat from commercial ice cream ranged from 27.40 to 31.35 with an arithmetical mean Reichert-Meissl number of 29.19 (Table 1). Results indicate that 160 (94.6%) of 169 samples had numbers between 28.0 and 30.5. Since a frequency distribution curve drawn from these data was slightly skewed, the median Reichert-Meissl number was also determined and was found to be 29.17.

TABLE 1

Reichert-Meissl number distribution of 169 samples of late summer butterfat

REICHERT-MEISSL NUMBER RANGE	NO. OF SAMPLES	% OF SAMPLES	% OF SAMPLES 0 10. 20. 30. 40.
0-27.0	0	0	
27.0-27.5	1	0.6	
27.5-28.0	2	1.2	
28.0-28.5	19	11.3	
28.5-29.0	35	20.7	
29.0-29.5	70	41.4	
29.5-30.0	24	14.2	
30.0-30.5	12	7.0	
30.5-31.0	5	3.0	
31.0-31.5	1	0.6	
ABOVE 31.5	0	0	

The arithmetical mean Reichert-Meissl number of butterfat from 105 commercial samples of butter was 28.40, with the lowest, 27.05 and the highest, 30.10 (Figure 1). These data are slightly lower than those in Table 1. The butter samples were collected from April 15, 1953, to March 15, 1954, which was a period of general drought and extreme feeding conditions in Kansas, whereas the ice cream samples were collected in a previous year when pasture and feeding conditions were good. The peak Reichert-Meissl numbers of the butter samples collected in April may be attributed to winter wheat or rye pasture, but no explanation is offered for the peak Reichert-Meissl numbers in July.

These data are similar to ranges of Reichert-Meissl numbers reported previously *(5, 12, 13, 14)*. Spitzer and Epple *(13)* found an average Reichert-Meissl

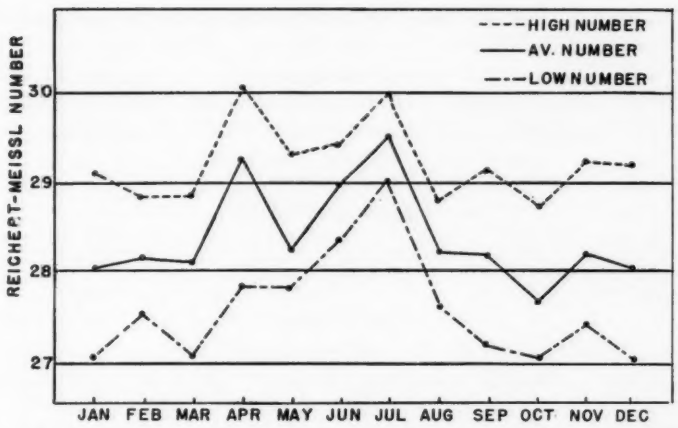

FIG. 1. Seasonal variation of Reichert-Meissl numbers of butterfat from 105 samples of commercial butter.

number of 29.91 for 500 samples of creamery butter produced during all seasons over a 4- to 5-year period. They reported the lowest Reichert-Meissl number was 26.45 and the highest, 32.10. Cox and McDowall *(5)* reported an average Reichert-Meissl number of 30.4 for 862 samples of New Zealand butterfat obtained from commercial butter over a 4-year period. A definite seasonal trend was observed. The data of Stout and Wilster *(14)* indicate that the Reichert-Meissl numbers of butterfat produced in three sections of Oregon during January varied with the type ration fed in each section and therefore tended to be independent of the season. The average Reichert-Meissl number of butterfat of each of three sections where different feeding conditions existed was 27.02, 30.65, and 31.10. However, the lowest and highest numbers found in any area in the state were 26.03 and 31.45. Butterfat having the lowest Reichert-Meissl values was produced in a region where alfalfa hay formed the major portion of the ration. It is recognized that feed influences butterfat composition and inherent glyceride composition. There are numerous references in the literature about the effect of various rations on the Reichert-Meissl number of butterfat *(1, 4, 6, 7, 8, 12, 15)*. In some instances extremely low Reichert-Meissl numbers have been obtained *(1, 8, 15)*. These were invariably caused by extreme or unusual feeding conditions, and frequently the butterfat samples were obtained from only a few animals. There is evidence in the literature that Reichert-Meissl values of butterfat, as found in commercial dairy products in certain states or regions, generally do not vary so much as the present accepted range. The results reported here tend to support that evidence.

SUMMARY

During August Reichert-Meissl numbers of butterfat from 169 samples of ice cream were determined. The arithmetical mean value was 29.19 and the range

was 27.40 to 31.35. Reichert-Meissl numbers of 160 of the 169 samples (94.6%) were between 28.00 and 30.50.

Over a 1-year period, 105 samples of commercial butter from 10 butter-producing plants in Kansas were analyzed. Samples were collected monthly. The arithmetical mean Reichert-Meissl number was 28.40 and the range was 27.05 to 30.10.

ACKNOWLEDGMENT

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AN APPRAISAL OF RESEARCH LITERATURE DEALING WITH LOOSE AND CONVENTIONAL DAIRY CATTLE HOUSING: A REVIEW[1]

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One of the dairy farmer's problems is to obtain low-cost housing which serves the enterprise effectively by minimizing production costs, accommodating an optimum size herd, and providing facilities for the production of high-grade products. Proponents of both loose housing and conventional barns have claimed that their respective systems satisfy these requirements.

This review was undertaken to ascertain the degree to which reported research answers questions that have arisen concerning the relative merits of loose and conventional housing. An effort was made to obtain a complete record of all reported research. Approximately 140 references relating to the subject were collected and reviewed. Many were extension type publications and popular articles not reporting new research. Several research references among the 140 were unavailable to the authors. Others were not cited because the projects did not deal specifically with the loose and conventional housing problem. Sixty-one publications were found that did report results of actual research. This article is based on these references, listed in the accompanying bibliography.

In order to study the literature systematically, an outline of questions was developed which encompassed the following phases of the problem: milk production, feed consumption, bedding requirements, cleanliness, dairy chore labor, herd health, and management practices. The literature was then reviewed and classified according to phases of the problem reported. Findings reported in different publications and relating to a specific question within the problem outline were then compared for similarity and consistency. When findings of several workers could not be compared directly, the authors of this paper made adjustments in the reported figures in order to place findings on a comparable basis. The degree of agreement of different investigations on various phases of the problem was considered a measure of substantiation.

This paper is divided into five major sections, each dealing with a problem area.

CONSTRUCTION COST

Buckley and Lamson *(8)* built two barns in 1913. They found that construction costs of an open stable were less than costs of a similar closed stable. Reduced expenditures for doors, walls, windows, and frames accounted for the

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savings. Brown *et al. (4, 5, 6)* visited 50 southern Michigan farms in 1948. Estimates for construction costs of conventional stanchion barns, warm loose-housing, cold loose-housing, and pole-frame loose-housing structures were $517, $441, $403, and $364 per cow, respectively. Costs were based on 22-cow capacity barns and 1948 price levels.

A similar study by Byers *(10, 11, 12, 13)*, beginning in 1947, covered 48 Kentucky dairy barns. Estimates were based on a 20-cow herd and included costs of the barn, milking area, milk room, and silo at 1951 prices. Conventional stanchion barns, loose-housing pole-frame barns with tandem elevated milking stalls, and loose-housing pole-frame barns with abreast floor level milking stalls cost $408, $358, and $303 per cow, respectively.

In 1950, Boyd and Clark *(3)* interviewed New York state operators of 87 loose-housing barns. Eight of the barns were built since 1947. Costs ranged from $146 per cow for a poorly constructed pole barn to $492 per cow for a well-constructed, two-story loose-housing barn. Four conventional frame loose-housing barns averaged $400 per cow. Seventy-nine of the barns had been converted from conventional structures. Conversion costs averaged $1,799 per farm for material, hired labor, and farm labor. An additional $442 per farm was required for new milking area equipment. Fourteen per cent of 89 respondents listed reduced construction costs of loose housing as an advantage. None listed construction costs as a disadvantage. Thirty-five per cent reported adopting loose housing because it seemed to be the cheapest method of providing extra barn space or housing for a new dairy enterprise.

Kalbfleisch *et al. (34)* asked similar questions by mailed questionnaires to operators of loose housing in Canada in 1951. Reduced construction costs of loose-housing barns were listed as an advantage by 23% of the operators. Moser and Jarvesoo *(38)* studied 16 loose-housing barns in Massachusetts in 1951. Barn investments ranged from $25 to $750 per animal unit or from $18 to $150 when based on planned capacity. Lower investment was listed as a reasonably sure advantage of loose housing.

A survey of 350 Illinois dairy farms was reported by Van Arsdall *et al. (46, 47)* in 1953. The authors estimated that a 28-cow conventional stall barn with silo would cost $418 per cow relative to $267 for a comparable 30-cow loose-housing barn. Costs of remodeling a conventional barn into a 28-cow loose-housing system were estimated at $71 per cow.

Conclusions. Most of the cost comparisons were based on studies conducted from 1947 to 1951. Thus, loose-housing construction costs reported include many but not all features deemed essential. As design requirements for the various functional areas become standardized, additional cost comparisons may be necessary for consideration of such features. Work reported does include costs for items such as providing feeding areas, but not all of these conform to present design concepts.

Investigators generally agreed that initial construction costs for loose-housing barns were less than for conventional barns. Loose housing with pole-frame construction was least expensive.

Differences in barn type classifications used by research workers, in construction quality, in cost estimating procedure, and in price level caused wide variations in the absolute construction costs reported. This demonstrates the impracticality of presenting costs in absolute terms. Research should develop layouts and bills of materials for loose housing and conventional barns to assist farmers in making decisions on housing.

Several investigators suggested that loose housing was more adaptable and flexible than conventional barns with respect to other enterprises and changes in herd size. No information was reported on repair and maintenance costs of loose and conventional housing. Information on costs of remodeling conventional barns into loose-housing structures was limited.

FEED CONSUMPTION AND QUALITY MILK PRODUCTION

Fraser *(29)*, in 1905, and Kalbfleisch *et al. (34)*, in 1952, mailed questionnaires to operators of loose housing. Sixteen of 18 respondents in Illinois stated that cleaner milk was produced in loose housing. Nineteen per cent of the Canadian respondents to Kalbfleisch's questionnaire reported improved sanitation and milk quality in loose housing. Approximately 16% thought milk production per cow increased under loose housing.

Similar questions were asked in Boyd and Clark's survey *(3)* of New York loose housing operators in 1951. Twenty-four per cent of the respondents indicated increased milk production as one of the advantages of loose housing. None of the respondents listed lower milk production as a loose housing disadvantage. L. L. Boyd and Clark *(3)* obtained bacteria counts for each of 77 matched pairs of loose and conventionally housed herds. Bacteria counts were not significantly different.

Cargill and J. S. Boyd *(15)* sent questionnaires to 42 city health departments. They asked if milk produced in loose housing would be of equal, superior, or inferior quality to milk produced in conventional barns when handled by men of equal ability and sanitary habits. Sixty-four per cent of the respondents checked equal quality, 22% superior quality, and 14% reported they had no basis for judgment.

Buckley and Lamson *(8)* correlated temperature, milk production, feed consumption, and costs of production in a controlled experiment at Maryland from 1910-13. Cow groups were selected so as to be equal in weight, breed, and lactation period. Two groups were housed in a conventional barn and one in a loose-housing barn. The loose-housing group had free access to roughage. All cows received identical grain rations on the basis of individual production.

Lower feed costs were reported under loose housing in three out of four trials. The authors hypothesized that high feed cost of the one trial was caused by placing cows in loose housing for the first time in midwinter. The other trials began in early fall and cows made gradual environmental adjustments. There were no extreme differences in feed costs of producing 100 lb. of fat-corrected milk between groups housed in loose and conventional barns. However, the

groups in loose housing made slightly greater weight gains. According to Buckley, there were no material production decreases attributable to sudden temperature fluctuations unless cows were exposed to rain.

Buckley and Lamson compared fecal contamination of milk by making bacterial counts during the experiment. Counts were no greater for loose housing than for conventional housing. However, bacterial content of the milking room air was lower than that in the conventional barn. Lamson concluded that loose housing had a substantial advantage as a place to produce milk with low bacteria.

A controlled experiment, similar to the Maryland work, was conducted at The Pennsylvania State College *(18, 48, 49)* from 1910 to 1914. A loose-housed herd had keener appetites and consumed more roughage than one housed conventionally. Weight gain differences were within the range of experimental error. Neither barn had a significant advantage in the therms of feed consumed per pound of milk produced (Table 1). On the basis of temperature records, Davis concluded that milk yield of the loose-housed group decreased more rapidly each winter and that decreases were caused by low temperature.

Woodward's controlled experiment *(61)* at Beltsville in 1918 was similar to Buckley's except that cows were alternated from loose to conventional housing. Comparisons were made between an individual's record in each barn. Woodward reported that more TDN were consumed in the loose housing barn. However, there was little difference between barn types when comparisons of feed efficiency were based on his data (Table 1). Feed costs per hundredweight of FCM were $1.38 and $1.35 for the loose and conventionally housed groups, respectively. The feeding efficiency advantage reverted to loose housing when the records of a pair of timid cows were eliminated.

A series of controlled loose housing experiments was conducted at North Dakota by Dice *(19, 20, 21, 22)* from 1926 to 1945. His early work showed that cows exposed to low temperatures maintained production comparable to conventionally housed cows, provided they were adequately fed, given dry bedding, and sheltered from wind, snow, and rain. He concluded that loose-housed cows required no more nutrients for milk or butterfat production than cows housed in conventional barns. His loose housing had a slight feeding efficiency advantage (Table 1).

A dairy herd was placed in a loose-housing barn during 1937 at the University of Wyoming *(39, 40)*. This herd received the same amount of grain as a second herd which was conventionally housed. However, the loose-housing herd was given a little more hay. There was no difference in milk or butterfat yields, but the loose-housing group gained more weight.

Another controlled experiment to determine the relative effects of "pen" and conventional housing on milk production was conducted at Huntley, Montana, by Graves *et al. (30)* in 1940. The pen barn was similar to loose housing except that cows were kept in individual pens. The procedure was similar to previously described studies. Cows housed in the pen barn had small weight gain advantages. Graves' data showed that the conventional barn had a slight feed efficiency advantage (Table 1). He concluded that cows would produce

TABLE 1

Comparison of feed used in loose housing and conventional barns to produce 1 lb. of FCM

Investigator	Loose housing	Conventional barn	Loose housing requirement in terms of conventional barn requirement
	(lb. TDN)	*(lb. TDN)*	*(%)*
Davis	0.57[a]	0.56[a]	101.8
Woodward	0.71	0.68	104.9
Dice	0.71	0.75	94.7
Graves	0.53	0.52	101.9
Wisconsin	0.84	0.85	99.9

[a] Therms.

more milk and butterfat when permitted to run loose in a lounging or pen barn than if confined in stanchions.

The Wisconsin Controlled Dairy Barn Project *(17, 33, 52, 54, 55, 56, 57, 58, 59, 60)* began in 1940 and continued to 1951. The loose-housed herds used more TDN than those conventionally housed. Differences were due to increased roughage consumption. Feed efficiency was aproximately equal (Table 1). Feed costs in loose housing were 97% of the conventional barn feed costs. The loose-housing group gained more weight. Milk production and air temperature records gave no indication that production was affected by air temperature in either system.

Milk quality was tested through bacterial plate counts, sediment tests, odor tests, flavor tests, and quarter sampling. Milk from each barn was of satisfactory quality for fluid markets.

A controlled experiment designed to determine the effects of air temperature on milk production was conducted by Kelley and Rupel *(36)* at Wisconsin from 1930 to 1932. There was less difference than was expected between milk production in loose and conventional housing. Exposure did not reduce production except during extremely cold periods. In conjunction with the preceding work, Kelley *(35)* examined 100 Wisconsin dairy barns, noting the type and condition. All milk yields were obtained at receiving plants and checked with weather data. Comparisons indicated that barn construction could nullify adverse weather conditions. Cold barns with clean stable air, obtained without drafts, gave better results than warm, stuffy barns.

Lewis and Johnson *(37)* studied The Michigan State College loose-housed herd. A series of six 24-hour observations was made during the winter of 1952-53. Activity of each cow was recorded every 10 minutes. Loitering in the yard decreased on stormy days, but low temperature alone had little or no effect on cow activities.

Conclusions. Feed consumption–milk production research has been carried out by controlled experiments reasonably alike in design. Investigators report that more roughage was consumed by loose-housed herds, but differences were small in terms of TDN. Although some investigators found higher feed efficiencies in loose housing and others have found the reverse, the range of results is remarkably narrow. Feed efficiency appears to be about equal in both systems and thus

is apparently not related to barn type. Records showed that low air temperature had very little effect on milk production when cows were clean, dry, free from drafts, and free to exercise. Additional research on feed efficiency should utilize barns which conform to modern design standards and larger numbers of well-matched animals.

Differences in bacteriological quality of milk produced in loose and conventional housing were small. Satisfactory quality was obtained in both systems. Additional research may be necessary, however, as new types of equipment are developed.

BEDDING AND COW CLEANLINESS

Fraser's circular *(29)*, published in 1905, was the earliest report of bedding and cow cleanliness research. Twelve of 18 respondents to a mailed questionnaire indicated that more bedding was required in loose housing, and four thought there was no difference. Only one respondent stated that cows were dirtier and milk less sanitary. Kalbfleisch *et al. (34)*, in 1952, found that 30% of their respondents listed high bedding requirements as a loose-housing disadvantage. According to Davis *(18)*, who conducted a controlled experiment from 1910 to 1914, loose housing required more bedding but less labor to keep cows clean (Table 2). Cows were fed and milked on the bedded area.

TABLE 2

Bedding requirement comparisons

Investigator	Loose housing	Conventional barn	Comparison of requirements[a]	Bedding type
	(lb/cow/day)		*(%)*	
Davis	37.73	21.65	174	Sawdust
	25.50	8.68	291	Wood shavings
Woodward	8.30	4.94	168	—[c]
Graves	15-25	8-12	125-313	—
Brown	8-15	5-8	100-300	—
Wisconsin[b]	12.30	7.60	162	Straw
Byers	9.00	5.00	180	Straw
Stewart	6-7	—	—	Straw
Moser	5-25	—	—	—
Gray	17.18	6.06	283	—
	(tons/cow/year)			
Boyd	1.50	0.50	300	—

[a] Loose housing requirement in terms of conventional barn requirement.
[b] Several investigators contributed to this research.
[c] Indicates the investigator did not specify the type of bedding used.

In 1913 Buckley and Lamson *(8)* reported a similarly designed project. Although bedding was not weighed, the authors indicated there were no large differences in requirements for loose and conventional barns. Cows became less soiled with manure in loose housing.

Woodward *(61)* recorded bedding weights for 3 months in his 1918 controlled experiment. Loose-housed cows were bedded often enough to keep the area clean, which was almost daily. They remained cleaner and seemed more comfortable than the conventionally housed group, but more straw was required (Table 2).

In controlled experiments at North Dakota *(19, 20, 21, 22)* Dice reported one-third more bedding was required in loose housing. No reference was made to bedding type, management, or cow cleanliness.

Graves *et al. (30)* recorded bedding requirements in their pen barn experiment at Huntley, Montana, in 1940. Bedding was added daily. Manure was removed when it attained a depth of 2 ft. As long as enough bedding was used, the pen barn cows were fully as clean as those conventionally housed. Pen barns required more bedding (Table 2).

Brown *et al. (4, 5, 6)* used interview and time-travel techniques in studying 43 loose-housing barns in southern Michigan during 1948. Data had been collected on 17 conventional barns the previous year. Loose housing required more bedding (Table 2). The degree of cow cleanliness was not reported.

Bedding requirements of the Wisconsin Dairy Barn Experiment *(17, 33, 52, 54, 56, 57, 58, 59, 60)* varied with management methods. Straw was the main bedding material. During the first trial loose housing required 34 lb. per cow per day compared with 13 lb. in the conventional barn. Cows were fed on the bedded area during this trial. The feeding area was paved before the second trial began, and the daily bedding requirements of the loose and conventional barns were reduced to 13 and 4.5 lb., respectively, per cow. Loose-housed cows appeared to be cleaner than in the first trial. In trials from 1943 to 1951 bedding was added daily but was shaken up during the day. This procedure reduced requirements to 12.3 lb. per cow per day.

Byers *(10, 11, 13)* conducted a survey of 56 Kentucky dairy farms from 1948 to 1952. Loose housing required more bedding than conventional housing (Table 2). Byers reported that the location of feed racks and holding areas influenced bedding requirements and recommended placing them so as to minimize travel on bedded areas.

Stewart *(44)* examined 36 loose-housing systems in Missouri in 1950 by survey and time-travel study. Records of bedding requirements were kept on five farms. Only one maintained a satisfactory bedding condition. This operator used 6 lb. of bedding per cow per day. Stewart concluded that satisfactory loose-housing conditions could be maintained with 6 to 7 lb. of straw per cow per day.

Boyd and Clark *(3)*, in 1950, reported one third of the loose-housing operations surveyed used more than 2 tons of bedding per cow per year. The second third used between 1 and 2 tons, and the rest used less than 1 ton. Bedding area arrangement affected the bedding requirement. Operators who combined feeding and bedding areas used 25% more bedding than those who separated them.

Moser and Jarvesoo *(38)* visited 16 Massachusetts loose-housing barns in 1951. Farmers were using straw, hay, broom corn waste, sawdust, shavings, and wood waste as bedding. Eight farmers stated that more bedding was required in loose housing. They used from 5 to 21 lb. of bedding per animal unit per day.

Gray *(31)* obtained data on bedding requirements on five loose-housing systems and five conventional barns in Massachusetts in 1953. Daily requirements ranged from 11.5 to 30.2 lb. per cow milked in loose housing and from 3.6 to 8.2 lb. in conventional barns. Gray supported the claim that loose housing required

more bedding. However, he hypothesized that management practices and type of barn were equally important as factors affecting bedding requirements.

Conclusions. Research has shown that loose housing requires more bedding than conventional housing. Little agreement exists as to how much more bedding is required. Estimates range from 100 to 340% of the conventional barn requirement.

Layout of loose housing affects bedding requirements. Separate bedding and feeding areas have reduced requirements. Layouts should minimize cow travel on the bedding. Management methods also affect bedding requirements. Labor spent reducing bedding needs will not necessarily offset other labor advantages in loose housing.

Observations by farmers and investigators indicate that cows are cleaner in loose housing than in conventional barns. No controlled experiments have been designed to measure cleanliness. However, objective measurements have been approached through bacterial counts and herd health records. Bacterial counts and sediment tests have been treated in the previous sections. Milk has met market regulations when cows in loose-housing systems have been bedded adequately.

Bedding requirements are a serious disadvantage to loose housing in many areas. Farmers should determine their bedding supplies before beginning loose-housing operations. Further investigation is needed to determine bedding requirements in various areas and to find straw substitutes.

DAIRY CHORE LABOR

Dairy chore labor research began in 1905 with Fraser's questionnaire *(29)* to loose-housing operators. Labor saving was given as the chief advantage by 16 of 18 respondents.

According to 86% of the respondents to the questionnaire mailed in 1951 by Kalbfleisch *et al. (34)*, less labor was required in loose housing than in conventional barns. Operators estimated that loose housing saved 20 to 50% of the labor previously required in their conventional barns. The authors estimated that labor requirements could be reduced to 60 hours per cow per year in loose housing and to 70 hours in conventional barns.

Woodward's labor records *(61)*, compiled in his controlled experiment in 1918, indicated that preparing cows for milking and bedding them required more time in loose housing. Removing manure and cleaning the milking area required less time. Woodward concluded that loose housing required more labor than conventional housing, considering all operations except milking and feeding. According to Woodward, the latter chores were excluded from the comparison for they required equal time in both types. More recent investigations have shown that milking and feeding require less time in loose housing.

Rorholm and Engene *(41)* ran loose-housing barn time studies at the Rosemonte Research Center in Minnesota during 1952. Milking, feeding roughage, cleaning the milking area, and hauling manure required less time in loose than

in conventional housing. Caring for milk, milking equipment, and the milk house; feeding grain; and supplying the bedding took more time. Total dairy chore time was 137 hours per cow annually in loose compared with 145 hours in conventional barns.

In 1949, Sturrock *(45)* studied labor organizations on 140 farms in England. Survey records were divided into three groups; first, farms with cowsheds; second, yard and cowshed systems with bucket type milkers; and third, yard and parlor systems with walk-through milking rooms and releaser machines. Over-all chore times in the three systems averaged 182, 169, and 139 hours per cow per year, respectively. Yard and parlor time savings occurred in milking, cleaning, and feeding. Sturrock concluded that well-designed yard and parlor systems required less labor than cowshed systems but that individual management and layout were more important than barn type.

Farmers interviewed by Boyd and Clark *(3)* in 1950 estimated loose-housing chore labor requirements. Those with separate feeding and bedding areas averaged 11.6 hours per cow annually in cleaning with power loaders. Where feeding and bedding areas were combined, cleaning required 4.7 man-hours per cow per year. Bedding time averaged 3.2 hours per farm per week. Milking plus cleaning time averaged 5.5 minutes per cow per milking. When asked to list loose housing advantages, 63 of 89 respondents reported lower labor requirements.

According to Stewart *(44)*, considerable labor could be saved by using more effective chore labor techniques. In his 1950 Missouri time-travel study only three of 36 loose-housing operators practiced self-feeding of hay. He found that the number of cows milked per man-hour varied from 8.4 to 11.1 in elevated and floor-level milking rooms. He concluded there was little difference between milking rooms measurable in terms of time and that loose housing offered other chore labor advantages.

In 1948 Brown *et al. (4, 5, 6)* synthesized loose-housing dairy chore routines from measurements of time-travel. Synthesis involved dividing jobs into work elements, eliminating or combining elements, rearranging work areas, selecting efficient elements, and recombining them. Standards were based on slightly less than the best performance observed. The synthesized annual labor requirement per cow was 61 hours in loose housing and 70 hours in the conventional barn. Time savings were figured in herding, feeding, and cleaning.

Labor requirements of loose and conventional housing were investigated by time-travel methods in the Wisconsin Dairy Barn Study *(17, 52, 54, 56, 57, 58, 59, 60)*. In 1950-51 a loose-housing barn equipped with an elevated stall milking room with a three-unit pipeline milker and bulk tank was compared with a conventional barn where one man operated a single-unit bucket milker. Mechanical manure removers were used in loose housing, and hand methods were used in the conventional barn. Loose housing required from 30 to 35% less total chore time than the conventional barn. When hand manure-removal methods were used in both barns, total chore time was 8% less in loose than in conventional barns. The investigators pointed out that labor relationships varied with equip-

ment, operators, and routines and also that fatigue was not measurable by time alone.

A milking room study *(51, 53)* was conducted during 1949 in conjunction with the Wisconsin project. Labor requirements in four basic milking room arrangements were studied by time-travel techniques. Less time was required to milk cows in milking rooms than in conventional barns. Twenty to 25 cows were milked per man-hour under varying routines in the several milking rooms. Research on milking labor requirements, rates and routines was also conducted by Dodd *(23)* in England, Bettenany *(2)*, Cullity *(16)*, and Scott and Scott *(42)* in Australia, and by Whittleston *(50)* in New Zealand. These investigators reported that high milking rates and low labor requirements were characteristic of milking rooms.

Moser and Jarvesoo *(38)* questioned 16 Massachusetts loose housing and eight conventional barn operators on chore times in 1951. Estimates of daily total chore time averaged 13 minutes per cow for loose housing and 21.3 minutes for conventional barns. Operators reported that loose housing work was easier. It was concluded that labor savings were an advantage of loose housing.

Moser and Jarvesoo's work was followed in 1953 by Gray's time study *(31)* of five loose and five conventional housing systems in Massachusetts. Loose and conventional housing total chore time per cow per day averaged 13.5 and 19.9 minutes, respectively. Time was saved in milking, feeding, and barn cleaning. Gray concluded that loose housing saved labor but pointed out that layout and management were as important as barn type.

Elliot and Jenkins *(24)*, in 1951, conducted a time-travel study of 21 Nova Scotia dairy barns. Loose-housing dairy chore time averaged 32.5 minutes per cow per day compared with 31.4 minutes for the conventional barns. The authors indicated that loose housing was so new that operators lacked well-organized routines.

Byers *(9, 10, 11, 12, 13, 14)* conducted a time-travel study of 56 Kentucky dairy barns from 1948 to 1952 and synthesized chore labor requirements for several dairy housing systems. Dairy chores averaged 120 hours per cow annually in five conventional barns. Byers' synthesized routine for conventional barns required 77 hours per cow anually. Nine walk-in-back-out milking room rest shed systems averaged 146.6 hours per cow annually, but the synthesis for this type barn was 80.2 hours. The 16 rest shed operations with walk-through milking rooms averaged 75.2 hours per cow annually, but a synthesized requirement was 51.8 hours. The seven elevated milking room rest shed systems averaged 85.2 man-hours per cow per year against 54.1 for a synthesized routine.

Shute *(43)* in 1952 reported a farm survey and stop-watch study of 21 conventional and 33 loose housing barns. Survey data showed that loose housing required 72 man-hours per cow annually compared to 86.9 hours for conventional barns. In the stop-watch study, loose housing required 69.4 man-hours per cow annually, compared to 80.4 hours in conventional barns. All chores except feeding required less time in loose housing. Labor requirements were also synthesized by combining the most efficient chore routines. Synthesized routines required

TABLE 3

Summary of loose and conventional housing chore time comparisons

Chore	Number of comparisons[a]	Loose housing time relative to conventional barn time	Range	
			High	Low
		(%)	(%)	
Milking	10	84	97	62
Feeding	9	80	97	41
Bedding	11	86	175	25
Cleaning	10	80	144	45
All dairy chores[b]	14	79	96	61

[a] By different investigators of each chore in loose and conventional barns.
[b] Measured from the beginning to the end of dairy chores, not the total of milking, feeding, bedding, and cleaning.

54.9 and 73.2 hours per cow per year, respectively, for loose and conventional housing. Shute concluded that loose housing took less labor and less "physical ability."

Results of a time-travel study by Baker and Bailey *(1)* on 15 central Ohio farms were published in 1952. Five conventional barns were compared with five loose-housing barns. The annual loose-housing chore time was 98 hours per cow against 93 hours for conventional barns. Synthesized chore routines and labor requirements for a 20-cow herd were prepared. Winter labor estimates for loose and conventional housing were 246 and 251 minutes per herd per day, respectively. The summer labor estimates were equal.

Conclusions. Comparisons of labor requirements reported in the literature are complicated by variation in definitions, and findings of different investigators are not comparable in an absolute sense. However, each investigator's loose-housing results may be compared with his conventional barn results because definitions are usually comparable within an individual's research. Conclusions rest on the degree to which different investigators obtained similar results in their own barn type comparisons (Table 3).

Loose housing saves about 20% in terms of labor and 30% in terms of travel. Savings are made in milking, feeding, bedding, and cleaning time. Savings in cleaning are questionable because some investigators compared manual manure-handling methods for conventional barns with mechanical methods in loose housing. Travel savings were indicated for each job except bedding.

Labor was easier, less fatiguing, and took less physical stamina in loose housing with elevated milking rooms. The most general comment on working conditions was that loose-housing milking rooms were cold. Daily manure handling was reduced in loose housing, and chore labor, particularly feeding and cleaning, was more flexible, making labor organization easier.

Loose-housing milking rooms were studied by several investigators. Milking room design affects labor requirements. Floor-level walk-in-back-out milking rooms had relatively low milking rates in terms of cows milked per man-hour. Rates were highest for elevated-stall milking rooms. Well-designed milking rooms operated by one man with two milker units can handle 20 to 25 cows per man-hour.

ment, operators, and routines and also that fatigue was not measurable by time alone.

A milking room study *(51, 53)* was conducted during 1949 in conjunction with the Wisconsin project. Labor requirements in four basic milking room arrangements were studied by time-travel techniques. Less time was required to milk cows in milking rooms than in conventional barns. Twenty to 25 cows were milked per man-hour under varying routines in the several milking rooms. Research on milking labor requirements, rates and routines was also conducted by Dodd *(23)* in England, Bettenany *(2)*, Cullity *(16)*, and Scott and Scott *(42)* in Australia, and by Whittleston *(50)* in New Zealand. These investigators reported that high milking rates and low labor requirements were characteristic of milking rooms.

Moser and Jarvesoo *(38)* questioned 16 Massachusetts loose housing and eight conventional barn operators on chore times in 1951. Estimates of daily total chore time averaged 13 minutes per cow for loose housing and 21.3 minutes for conventional barns. Operators reported that loose housing work was easier. It was concluded that labor savings were an advantage of loose housing.

Moser and Jarvesoo's work was followed in 1953 by Gray's time study *(31)* of five loose and five conventional housing systems in Massachusetts. Loose and conventional housing total chore time per cow per day averaged 13.5 and 19.9 minutes, respectively. Time was saved in milking, feeding, and barn cleaning. Gray concluded that loose housing saved labor but pointed out that layout and management were as important as barn type.

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HERD HANDLING

"Boss" cows. The boss cow problem has been recognized by Woodward *(61)*, Boyd and Clark *(3)*, Kalbfleisch *et al. (34)*, Moser and Jarvesoo *(38)*, Byers *(10, 13)*, Brown *et al. (4, 6)*, and Witzel *et al. (56, 57, 58, 59)*. Investigators and the farmers interviewed agree that boss cows are more of a problem in loose housing. Farmer-survey results placed considerable emphasis on the problem. Investigators indicated that boss cow problems can be reduced by good loose-housing management. Dehorning, self-feeding hay in extra-long racks, tying, and selling were recommended as practices to reduce this problem.

Calf and maternity facilities. Groth and Esmay *(32)* recommended that in any barn calves be protected from sudden temperature fluctuations and provided with draft-free pens and dry bedding. Calves were born and raised in loose housing during the Wisconsin Dairy Barn Project *(17, 33, 54, 57, 58, 59)*. Calves born during low temperatures were warmed and dried. Considerable success was reported. Kalbfleisch *et al. (34)* recommended that breeding programs be planned to avoid calving during the two coldest months. However, 56% of the Canadian loose-housing operators surveyed allowed calves to be born and raised in loose housing regardless of weather. On the basis of an extensive controlled experiment, Erb *et al. (25, 26, 27)* concluded that dairy calves could be raised successfully in loose housing. Records of calves housed in an open shed were compared with those of calves housed in a conventional barn. Erb emphasized keeping bedding dry and protecting animals from excessive air movement. Investigators agree that dairy calves can be raised successfully in properly designed and managed loose-housing systems.

Individual attention. The fact that loose-housed cows receive less individual attention was brought out by Kalbfleisch *et al. (34)* and by Boyd and Clark *(3)*. Farmers reported that it was difficult in loose housing to control roughage feeding and to detect and treat cows off-feed, sick, or injured.

Although the feed control problem has not been studied extensively, the feed efficiency work indicated no large milk production efficiency differences between loose and conventionally housed herds. The problem of detecting cows off-feed, sick, or injured suggests placing more emphasis on everyday herd observation. This difficulty may be compensated in part by lower injury occurrence in loose housing.

Herd attractiveness. Loose-housed cows do not show as well as those in a conventional barn, according to Boyd and Clark *(3)*, the Wisconsin Dairy Barn Project *(17, 59)*, Byers *(10)*, and Kalbfleisch *et al. (34)*. Since cows were not lined up at any one time in loose housing, grooming was more difficult, and appearances less orderly.

Breeding and heat detection. Investigators in the Wisconsin project *(58, 59)* indicated that heat periods were more easily detected in loose housing. This was also reported in surveys by Boyd and Clark *(3)* and Kalbfleisch *et al (34)*. Some farmers believed that cows in heat disturbed the herd and that loose housing increased difficulties in handling animals for breeding or veterinary care.

Fly control. Methods of fly control were investigated in the Wisconsin Dairy Barn Project *(58)*. The best control was to remove the manure pack before the fly breeding season. When removal was not possible before this time it was recommended that cows be kept off the manure pack. This allowed a manure pack crust to develop and reduced fly breeding.

Fly control problems have not been referred to in any of the other reported research. Apparently the problem has been either minor or thought to be unrelated to barn type. Milk of satisfactory quality has been produced in both types of barns.

Injury and disease occurrence. Graves *et al. (30)*, Fourt *et al. (28)*, Heizer *et al. (33)*, Witzel *et al. (17, 54, 58, 59)*, and Kelley and Rupel *(36)* kept records of injury occurrence in controlled loose and conventional housing experiments. Lower incidences of injuries such as udder damage, lameness, and sore feet were reported in loose housing by everyone except Kelley and Rupel. Woodward *(61)* proposed that there were no great differences in the incidence of injuries in the two barns. The seriousness of injuries was evaluated in the Wisconsin Project *(58)* by observing the length of production lags. Reduced injury occurrence was advanced as a loose-housing advantage by high proportions of the respondents to the surveys of Moser and Jarvesoo *(38)*, Boyd and Clark *(3)*, and Kalbfleisch *et al. (34)*.

Few experiments were designed to measure disease occurrence. Woodward *(61)* reported no differences between loose and conventional housing. Barn type was reported to have little effect on mastitis in the Wisconsin study. Dice *(20, 21)* and Graves *et al. (30)* reported more mastitis in loose housing. Graves *et al.* pointed out that this was not a result of loose-housing conditions. Bryan *(7)* kept herd histories and treated mastitis cases in three loose-housing barns. He reported that mastitis could be controlled by proper management and veterinary service. The surveys of Moser; Boyd and Clark; and Kalbfleisch *et al.* agree that farmers reported less mastitis and that cows were healthier in loose housing.

Most of the injury and disease occurrence research has been based on comparisons of loose housing and conventional barns with stanchions. The problem should be studied further by using comfort stalls and comparing the seriousness of the maladies.

SUMMARY

This examination of research on dairy cattle housing has brought out the points for and against loose housing relative to conventional barns. On some farms loose housing may be a solution to problems of labor efficiency and building expansion. Loose housing, however, is not the solution for every operator, even though it appears to be a low-cost, efficient, functional housing unit. Selection of the type of barn must depend on the relative advantages and disadvantages of alternative barn systems in relation to particular farm situations.

Conclusions evaluating the various phases of the loose and conventional housing question are presented either as a part of the research cited or immediately following each section of this paper.

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THE VALUE OF CERTAIN SURFACTANTS AND OTHER GROWTH STIMULANTS IN THE RATIONS OF YOUNG DAIRY CALVES[1]

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Ely *(5)* in 1951 and Ely and Schott *(6)* in 1952 found in a routine toxicity study that certain surface active agents appeared to stimulate growth in chicks. Luecke *et al. (10)* in 1952 found that the addition of 0.1% Ethomid C/15 to swine rations increased the daily rate of gain significantly and also improved feed efficiency. Since these early reports, various workers *(1, 2, 4, 11, 13, 14, 16)* experimenting with chicks or swine have either confirmed or have failed to confirm the earlier observations of Ely *(5)* and Luecke *et al. (10)*.

Gardner *et al. (7)* reported that the addition of Ethomid C/15 to the diets of young calves on ad libitum milk feeding did not stimulate growth, whereas the addition of aureomycin did improve the growth rate. The evidence of scours was much greater in the Ethomid C/15 supplemented group. Hyde and Rusoff *(8)* reported that the addition of anionic, cationic, and nonionic surface active agents to the diets of young dairy calves produced no beneficial results and even caused losses in weight, produced a rough appearance, and reduced feed consumption during the latter stages of the trials. Voelker *et al. (17)* found that young dairy calves receiving rations supplemented with five different surfactants made similar gains as their controls but scoured a total of 110 days as compared to 43 days for the controls. In a later study Voelker and Jacobson *(18)* found that young dairy calves fed aureomycin gained 120% as much as the controls, whereas the group supplemented with a detergent gained only 90% as much as the controls. Lassiter *et al. (9)* reported preliminary results which indicated that Ethomid C/15 alone or in combination with Arquad HT stimulated the growth rate of young dairy calves raised on a limited milk-starter-hay system.

The purpose of the studies reported here was to evaluate various surfactants and other growth stimulants in the rations of young dairy calves.

The results of two experiments, designated as Experiments I and II, are reported concerning the feeding value of various surfactants in the rations of young dairy calves. Part of the results of Experiment I were reported previously *(9)*.

EXPERIMENT I

Procedure. Thirty-six Jersey and Holstein male and female calves were evenly divided into six groups and raised to 86 days of age on a limited milk-

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starter-hay feeding program. The calves were permitted to remain with their dams for 48 hours after birth. Each calf was then placed in an individual pen, weighed, and subjected to an experimental regime of limited whole milk feeding along with free choice feeding of good quality alfalfa hay and water.

A simple calf starter composed of 100 lb. of corn distillers' solubles, 120 lb. crimped oats, 80 lb. ground corn, 80 lb. wheat bran, 50 lb. linseed oil meal, 5 lb. steamed bone meal, 5 lb. iodized salt, and 200 g. of a vitamin A and D concentrate was fed ad libitum to a maximum of 4 lb. daily per calf. This starter contained 17.9% crude protein and 6.4% crude fiber. Hay and grain feeding was started at 1 week of age. The daily whole milk feeding schedule was as follows: 0-2 days of age—colostrum; 2-21 days—8% of body weight; 22-28 days—6%; 29-35 days—5%; 36-42 days—3%; 43-49 days—2%; 50 days and over—no milk.

Group 1 was considered the control group and was not supplemented. Group 2 was fed an aureomycin supplement (Aurofac 2A) at the rate of 24 mg. per pound of starter; Group 3 was supplemented with a nonionic surface active agent, Ethomid C/15,[3] at the rate of 0.1% of the starter; Group 4 was fed a cationic surfactant, Arquad HT,[4] at the level of 0.025% of the starter; Group 5 was fed a combination of Ethomid C/15 and Arquad HT at one-half the levels fed to Groups 3 and 4; Group 6 was fed a combination of Ethomid C/15 and aureomycin supplement at the same rates as Groups 2 and 3. For the first 49 days all supplements were fed in the milk. An aureomycin supplement (Aurofac D) was fed at the rate of 75 mg. daily, Ethomid C/15 was fed at the rate of 1 g. daily alone or in combination with aureomycin and at the rate of 0.5 g. when fed with Arquad HT, and Arquad HT was fed at the rate of 0.25 g. daily when fed alone and 0.125 g. when fed with Ethomid C/15.

Feed consumption records, observations of the incidence of scours, and overall condition of the animals were recorded daily. The calves were weighed weekly, and height at withers and chest circumference measurements were taken monthly.

Results. A summary of the effect of the surfactants and aureomycin on the daily gains by ages and breeds is presented in Table 1. An analysis of these data by covariance *(15)* indicated a significant difference between the growth rate of the basal group and of each supplemented group, except Group 2, at the 5% level of probability. At the 1% level of probability only the gains of Groups 3 and 6 were significantly greater than those of Group 1. The growth of the calves in Group 6 was also significantly greater than that of the calves in Groups 2 and 4 at this level. The addition of the aureomycin supplement to the basal ration increased the daily rate of gain, but this increase was not statistically significant. All of the surfactants studied stimulated the growth of the calves, but there were some variations between surfactants. Ethomid C/15 appeared to have a greater effect on the rate of gain than Arquad HT. A combination of these two produced an intermediate effect. When aureomycin was combined

[3] Ethomid C/15 is polyoxyethylene cocoamide.

[4] Arquad HT is tri-methyl octadecyl ammonium chloride.

TABLE 1

Effect of surfactants and aureomycin on the daily gains of Holstein and Jersey calves

Group		Weeks								
		0-4			0-8			0-12		
		Holstein	Jersey	Group av.	Holstein	Jersey	Group av.	Holstein	Jersey	Group av.
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
1	Basal	0.57	0.61	0.59	0.88	0.79	0.83	0.95	0.88	0.91
2	Basal plus aureomycin	0.67	0.63	0.65	0.73	0.84	0.79	1.13	0.94	1.04
3	Basal plus Ethomid C/15	0.70	0.37	0.54	1.13	0.73	0.93	1.43	0.98	1.21
4	Basal plus Arquad HT	0.62	0.19	0.40	0.96	0.57	0.76	1.35	0.82	1.09
5	Basal plus Ethomid C/15 plus Arquad HT	0.74	0.33	0.53	1.14	0.70	0.92	1.41	0.84	1.13
6	Basal plus Ethomid C/15 plus aureomycin	0.58	0.51	0.55	1.08	0.89	0.99	1.54	1.11	1.32

with Ethomid C/15 a greater growth response was observed than with either one alone. Apparently there was a supplemental effect between these two growth stimulants. Aureomycin and both of the surface active agents appeared to have a greater effect on the growth rate of Holstein calves than on that of Jersey calves. It should be pointed out that these breed differences should be evaluated in the light that they represent only three calves of each breed. The two surfactants alone or in combination appeared to have a depressing effect upon the growth of Jersey calves under 4 weeks of age. This fact is difficult to explain unless an excessive amount of surfactant was fed to the Jersey calves. All supplements for the first 49 days were fed on a per-calf basis rather than on a body-weight basis. The various supplements affected skeletal growth, as measured by the increase in height at withers and chest circumference, about the same as they did average daily gain.

The incidence of scours was much greater among the calves of Group 4, calves fed Arquad HT alone, than among the calves in any of the other groups. The incidence of scours quoted as total number of scouring days per group was 14, 0, 11, 27, 8, and 4 for Groups 1, 2, 3, 4, 5, and 6, respectively. Apparently, aureomycin alone or in combination with Ethomid C/15 tended to reduce the incidence of scours. Ethomid C/15 alone or in combination with Arquad HT had little effect on the incidence of scours. When Arquad HT was fed alone, however, the incidence of scours was markedly increased. This was particularly evident during the second and third weeks of life. The scours usually could be cured by the administration of sulfa drugs for 3 or 4 days.

A summary of the feed consumption and feed efficiency data is presented in Table 2. The addition of both surfactants and aureomycin to the basal ration alone or in combination with each other was associated with an increase in consumption of feed and an improvement in the efficiency of feed conversion. The combination of Ethomid C/15 and aureomycin appeared to exert the greatest influence on the consumption of feed and feed efficiency. These data would indicate a relationship between the effect an antibiotic or surfactant has on the consumption of feed and the growth response received from these supplements. It should be pointed out that although there was some improvement in the efficiency of feed utilization, these differences were not statistically significant.

TABLE 2

Summary of feed consumption and efficiency of feed utilization data to 12 weeks of age

Group		Average total consumption			Feed/gain
		Milk	Starter	Hay	
		(lb.)	*(lb.)*	*(lb.)*	*(lb/lb)*
1	Basal	217.7	113.4	108.1	3.25
2	Basal plus aureomycin	210.6	120.8	106.8	2.91
3	Basal plus Ethomid C/15	221.6	144.8	114.6	2.83
4	Basal plus Arquad HT	209.1	125.5	114.4	2.91
5	Basal plus Ethomid C/15 plus Arquad HT	204.6	117.4	143.5	3.03
6	Basal plus Ethomid C/15 plus aureomycin	197.9	167.1	119.1	2.80

None of the supplements appeared to have any appreciable effect on the condition of the animals, with the possible exception of the combination of Ethomid C/15 and aureomycin. The calves fed this combination, particularly the Jerseys, appeared to be healthier and more active, and their hair coats possessed more bloom.

EXPERIMENT II

Procedure. Forty-eight Jersey and Holstein male and female calves were divided equally into six groups. The feeding and management of the calves were essentially the same as outlined in the first experiment. The only change in the feeding program was that soybean oil meal replaced linseed oil meal in the calf starters. The experimental groups were as follows: Group 1—control; Group 2—basal plus an aureomycin supplement, fed at the rate of 24 mg. of aureomycin per pound of starter; Group 3—basal plus Ethomid C/15 at the rate of 0.1% of the diet; Group 4—basal plus 100 g. per ton of an insoluble organic salt of a cationic surfactant (tri-methylalkyl ammonium stearate); Group 5—basal plus Arquad HT at the level of 25 g. per ton; Group 6—basal plus a combination of Ethomid C/15 and aureomycin at the same levels as for Groups 2 and 3. Tri-methylalkyl ammonium stearate and Arquad HT were fed at the rates of 0.1 g. and 0.032 g. per day, respectively, for the first 49 days. All other supplements were fed at the same rate as in Experiment I. Arquad HT was fed at a lower level than in the first experiment in an attempt to reduce the incidence of scours of calves fed this surface-active agent.

Results. The effect of the various supplements on growth are summarized in Table 3.

An analysis of covariance *(15)* indicated there were no significant differences in the growth rate of the calves. The differences between Groups 1 and 2 or 6 were approaching significance at the 5% level of probability.

In this study neither one of the surfactants nor tri-methylalkyl ammonium stearate stimulated the growth rate of the calves. This was in contrast to the findings in Experiment I. Neither was there an added growth response obtained when Ethomid C/15 was fed in combination with aureomycin. In this study all of the growth response appeared to be due to the influence of aureomycin. The growth depression of Arquad HT in Jersey calves for the first 8 weeks was not observed, as in Experiment I. This could possibly be attributed to the lower level fed in this trial. Tri-methylalkyl ammonium stearate, however, did appear to depress the growth of Jersey calves for the first 8 weeks. It did not seem to have any effect on the growth rate of Holstein calves.

The incidence of scours was not a problem in this study except with the calves fed tri-methylalkyl ammonium stearate. Total number of scouring days per group for all calves was 15, 12, 17, 39, 12, and 9 for Groups 1, 2, 3, 4, 5, and 6, respectively. The incidence in Group 4 was extremely high when the calves were between 2 and 4 weeks of age. Six out of the eight calves in this group scoured a total of 4 to 11 days. Higher levels of tri-methylalkyl ammonium stearate ranging from 100-1600 g. per ton have been fed, but these higher levels produced

TABLE 3

Effect of the surfactant and other supplements on average daily gain of calves in Experiment II

Group		Weeks								
		0-4			0-8			0-12		
		Holstein	Jersey	Group av.	Holstein	Jersey	Group av.	Holstein	Jersey	Group av.
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
1	Basal	0.56	0.34	0.45	0.93	0.78	0.85	1.29	0.92	1.10
2	Basal plus aureomycin	0.64	0.27	0.45	1.16	0.76	0.96	1.45	0.99	1.22
3	Basal plus Ethomid C/15	0.56	0.33	0.45	0.88	0.73	0.81	1.16	0.98	1.08
4	Basal plus tri-methylalkyl ammonium stearate	0.68	0.24	0.46	1.04	0.56	0.80	1.35	0.86	1.11
5	Basal plus Arquad HT	0.49	0.24	0.36	0.92	0.67	0.79	1.26	0.96	1.11
6	Basal plus Ethomid C/15 plus aureomycin	0.72	0.21	0.47	1.15	0.67	0.91	1.45	0.98	1.22

no beneficial effects on the incidence of scours. Aureomycin was the only supplement which caused an increase in skeletal growth, and this effect was similar to the increase in body weight. The feed consumption and feed efficiency data are presented in Table 4.

TABLE 4

Summary of feed consumption and efficiency of feed utilization data of calves in Experiment II

Group		Average total consumption			Feed/gain
		Milk	Starter	Hay	
		(lb.)	*(lb.)*	*(lb.)*	*(lb/lb)*
1	Basal	212.2	147.0	68.4	2.60
2	Basal plus aureomycin	212.7	166.2	68.0	2.54
3	Basal plus Ethomid C/15	206.9	133.0	87.3	2.73
4	Basal plus tri-methylalkyl ammonium stearate	230.8	124.9	121.4	2.96
5	Basal plus Arquad HT	218.6	145.0	76.0	2.66
6	Basal plus Ethomid C/15 plus aureomycin	203.2	151.5	75.9	2.47

Aureomycin alone or in combination with Ethomid C/15 appeared to be the only supplement that had any appreciable effect upon feed consumption. The surfactant-fed calves and calves fed tri-methylalkyl ammonium stearate tended to consume slightly more hay and less starter than the control calves. This was particularly evident when the basal ration was supplemented with tri-methylalkyl ammonium stearate. The hay consumption of this group was much higher than that of the other groups. Feed efficiency of calves in Groups 2 and 6 was improved over that of the basal calves, showing again a close relationship between feed consumption, feed efficiency, and average daily gain of aureomycin-fed calves. In general, the feed efficiency of the surfactant calves was poorer than that of any of the other groups. Possibly this was due to the greater consumption of hay and less of starter by these calves.

Slaughter studies were made of representative calves from each group. All of the carcasses appeared to be of equal quality, and no abnormalities were observed in the livers, kidneys, hearts, or lungs of any of the calves. Surfactants or the other growth stimulants, therefore, do not appear to have any harmful effects on the internal organs of calves of the ages covered in these trials and at the levels that the supplements were fed.

The improved condition of the animals fed the basal ration supplemented with the combination of Ethomid C/15 and aureomycin was not observed in this study as had been the case in Experiment I.

DISCUSSION

In the present investigation it was shown that certain surfactants do stimulate the growth rate of young dairy calves under certain feeding regimes but do not under others. It is difficult to explain the differences in the response obtained from the feeding of surfactants in the two experiments. The only difference in the feeding and management of the calves was that during the second experiment a basal calf starter was fed which contained soybean oil meal instead of the linseed oil meal employed in the first experiment. When this change was made it

meant that the type of the plant protein was changed and that there was a slight increase in the crude protein content. The starters used in Experiment I contained about 17.9% protein, whereas the protein content of the starters used in Experiment II averaged 21.5%. It is possible that the change in the type of plant protein was responsible for the lack of response from surfactants in the second trial. Rusoff *et al. (12)* have reported a difference in the response to aureomycin depending upon the type of cottonseed oil meal or soybean oil meal fed to young calves.

It is possible that the year in which the two experiments were conducted could have exerted an influence on the response from surfactants, since the two studies were conducted during the fall and winter months of the years 1952-53 and 1953-54. The type of environment present during the two years possibly exerted an influence on the results obtained from the feeding of surface active agents. A portion of the calf housing facilities was destroyed by fire immediately after the first experiment was completed and was rebuilt immediately before the start of Experiment II. The effect of clean environment has been demonstrated *(3)* in the response of chicks to antibiotics. This explanation, however, does not seem justified, since a response from aureomycin was obtained in the second trial.

Based upon the results of these studies, it does not appear that the feeding of the surfactants used in these studies to young dairy calves can be justified at this time. The growth responses obtained from the feeding of surfactants are very erratic, probably influenced by many factors, and surfactants do not appear to have any beneficial effects on the incidence of scours and in some cases appear to increase the incidence of scouring in young dairy calves. Tri-methylalkyl ammonium stearate also increases the incidence of scours significantly.

SUMMARY

Eighty-four Holstein and Jersey calves were used in two experiments to evaluate certain surfactants and other growth stimulants in the rations of young dairy calves. All of the calves were started on experiment at 2 days of age and raised to 86 days of age on a limited milk-starter-hay system.

It was found in the first experiment that the surfactants Ethomid C/15 and Arquad HT alone or in combination stimulated the growth of the calves. An aureomycin supplement or aureomycin plus Ethomid C/15 also improved the growth rate of the calves. In the second experiment neither surfactant fed, Ethomid C/15 or Arquad HT, nor tri-methylalkyl ammonium stearate stimulated growth, but an aureomycin supplement, as well as a combination of aureomycin and Ethomid C/15, did. The combination, however, did not produce any greater growth response than aureomycin alone, thus indicating no supplemental effect of the surfactant on the antibiotic. Neither of the surfactants nor tri-methylalkyl ammonium stearate had any beneficial effects on calf scours and in some cases increased the incidence of scours, especially tri-methylalkyl ammonium stearate.

These data indicate that the feeding of surfactants used in these studies or tri-methylalkyl ammonium stearate to young dairy calves does not appear to be

justified at this time. The growth responses obtained were very erratic and were probably influenced by many factors. Possible explanations of the reasons for these variable results are discussed.

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ALFALFA VS. PRAIRIE HAY FOR DAIRY CALVES[1]

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Previous work *(5, 6, 7, 8, 9, 10,* and *12)* has shown that dairy calves will thrive on legume, mixed legume and grass, and nonlegume hays. Both legume and grass hays have been shown to be satisfactory roughages for the young calf.

It was the purpose of this experiment to determine the relative values of alfalfa and prairie grass hays for the young dairy calf *(a)* when these hays were offered at birth and *(b)* when they were not offered until the calf was 8 weeks of age.

EXPERIMENTAL PROCEDURE

Two 16-week feeding trials were conducted, each involving 20 Holstein and Jersey calves. The first trial began in mid-winter and carried over into the spring season. The second trial began in the summer and carried over into the fall season. In each trial there were four groups of five calves each. Group 1 was offered alfalfa hay ad lib. from birth; Group 2 was offered alfalfa hay ad lib. after the calves were 8 weeks of age; Group 3 was offered prairie hay ad lib. from birth; and Group 4 was offered prairie hay ad lib. after the calves were 8 weeks of age. In addition, each calf was fed up to 4 lb. of calf starter per day and an average total of 375 lb. of Holstein herd milk.

Calves were assigned at random, within breed, to one of the four groups. All calves were allowed to remain with their dams for 48 hours and then placed in individual solid-wall pens. Sawdust was used for bedding. All feeding was done twice daily and water was available in automatic water cups. Body weight, height at withers, and heart girth measurements were taken at weekly intervals. Blood samples for carotene and vitamin A analyses were taken at 2-week intervals after the calf was placed on trial. The method of Kimble *(2)* was used for blood plasma carotene and vitamin A. Carotene in hays was determined by saponification and phasic separation between petroleum ether and 90% methanol *(3)*. Proximate analyses of the hays and starter were made according to A.O.A.C. methods *(1)*, and Morrison's *(4)* digestion coefficients were used to make TDN calculations.

The data obtained at the end of the 4th, 10th, and 16th weeks of these trials were summarized separately. These intervals were chosen to evaluate the effect of treatment: *(a)* during early life; *(b)* at a time when all calves had had access to hay for 2 weeks or longer; and *(c)* for the entire experimental period.

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[1] A part of these data was presented by J. B. Williams in a thesis to the Graduate School, Oklahoma A. & M. College, in partial fulfillment of the requirements for the M.S. degree, 1953. Chemical analyses were made by R. J. Lloyd.

RESULTS AND DISCUSSION

A summary of the data is given in Table 1. The effect of treatment and season was evaluated by use of the analysis of variance *(11)*. There were no significant differences between treatment groups at the start of the trials, indicating good uniformity of body weight and size between and within treatments.

TABLE 1

Growth of calves, their feed consumption and efficiency of feed utilization in Trials 1 and 2

	Trial 1 (winter)				Trial 2 (summer)			
	Group				Group			
Items compared	1	2	3	4	1	2	3	4
Av. weight *(lb.)*								
Initial	80	72	64	85	67	70	81	71
4-wk.	101	84	84	99	86	88	96	83
10-wk.	159	133	139	149	130	133	131	118
16-wk.	185	189	173	170	226	194	198	219
Av. height at withers *(in.)*								
Initial	29.8	27.7	27.5	29.7	27.9	28.1	28.4	28.1
4-wk.	30.3	28.5	28.8	30.6	29.3	28.9	29.8	29.0
10-wk.	33.5	31.3	31.5	32.9	31.9	31.7	32.0	31.1
16-wk.	35.7	34.0	34.2	33.6	34.8	34.0	34.2	33.6
Av. heart girth *(in.)*								
Initial	30.4	28.4	28.3	30.7	29.0	30.0	30.3	29.5
4-wk.	32.0	29.4	30.3	31.2	31.5	31.5	31.7	39.6
10-wk.	37.9	34.9	35.4	36.3	36.3	36.0	36.0	34.8
16-wk.	42.4	40.0	40.0	41.4	40.6	39.6	39.4	38.4
Av. total feed consumption								
Starter *(lb.)*	276	269	266	291	227	232	254	234
Hay *(lb.)*	157	106	126	106	249	141	201	129
TDN *(lb.)*	373	338	346	355	386	328	376	321
Carotene *(mg.)*	2461	2095	1379	1605	4602	2932	2780	1938
TDN per lb. of gain *(lb.)*	2.6	2.8	2.7	2.8	2.3	2.7	4.2	3.3
Av. plasma composition *(γ per 100 ml.)*								
4-wk. carotene	11.3	33.1	25.1	19.8	19.2	24.1	14.3	21.0
10-wk. carotene	55.8	88.6	88.9	117.0	122.6	115.6	89.0	59.6
16-wk. carotene	77.3	113.0	98.3	176.1	174.4	117.5	116.0	130.0
4-wk. vitamin A	1.9	6.3	4.3	2.8	4.7	6.3	5.4	5.5
10-wk. vitamin A	4.8	5.3	6.2	5.5	7.0	5.1	6.2	5.8
16-wk. vitamin A	7.8	8.6	9.6	7.2	12.2	8.0	8.4	8.9

Calves started on trial in the winter (Trial 1) consumed significantly more starter ($P < 0.01$), gained significantly more ($P < 0.01$), and required significantly less ($P < 0.01$) TDN per pound of gain over the 16-week period than calves started on trial in the summer (Trial 2). Further, the winter-fed calves weighed more ($P < 0.05$) and had a greater heart girth ($P < 0.05$) at 16 weeks of age than the summer-fed calves. Carotene intake was less ($P < 0.01$) for the winter-fed calves, in part because of the fact that the hays compared in the winter trial had been in storage several months, whereas the hays compared in the summer trial were newly harvested. Differences in other measurements, including height at withers, and in plasma carotene and vitamin A were unrelated to season.

The main point derived from this experiment was found in the data dealing with the pounds of TDN required to produce a pound of gain for the 16-week period. There was a significant difference ($P < 0.01$) here due to treatment, season, and treatment-season interaction. Groups 1 and 2 required significantly less TDN per pound of gain than did Groups 3 and 4, respectively. There was no real difference between Groups 1 and 2 in this respect, indicating that calves will do as well when alfalfa hay is offered from birth as when it is withheld for the first 8 weeks of life. The difference in performance between Groups 3 and 4 was real, with Group 4 requiring less TDN per pound of gain than Group 3.

The data were regrouped to test further the above results. Treatment, season, and treatment-season interaction were significant ($P < 0.01$) when type of hay fed was the only consideration. When the only consideration tested was the age at which hay was fed, regardless of its kind, season alone was important ($P < 0.01$). This further suggests the important factor involved is that calves fed alfalfa hay will require less TDN per pound of gain than those fed prairie hay. Whether hay was fed from birth or after 8 weeks made little or no difference in terms of efficiency of TDN utilization, under the conditions of this experiment. These calculated TDN values may have either increased, depressed, or had no effect on the observed differences.

The consumption of carotene for the 16-week period was affected significantly ($P < 0.01$) by treatment. Calves receiving alfalfa hay from birth had a real advantage over calves in all the other groups in this respect. Group 1 consumed significantly more carotene than Group 2, and Group 2 consumed significantly more than either Group 3 or 4. There was no difference between Groups 3 and 4 in this respect.

Calves in Groups 1 and 3 had greater increases ($P < 0.05$) in heart girth at 4 and 10 weeks of age than those in Groups 2 and 4. There were no real differences between groups at 16 weeks of age. Thus, a temporary advantage was obtained in early body development by offering hay from the time of birth.

Blood plasma vitamin A and carotene values for all groups were erratic. With the exception of the plasma carotene levels at 4 weeks of age, there was more variation within groups than between groups. At 4 weeks of age the plasma carotene level of Group 2 was higher ($P < 0.05$) than that of Groups 1, 3, and 4. Since this situation did not continue, little value was attached to it.

There were no significant differences in body weight or gain in body weight between groups due to treatment at 4, 10, or 16 weeks; total starter consumption or total hay consumption per calf; TDN consumption per calf for the 16-week period; heart girth at 4, 10, or 16 weeks; blood plasma carotene at 10 and 16 weeks; blood plasma vitamin A levels at 4, 10, or 16 weeks; or height at withers at 4, 10, or 16 weeks.

SUMMARY

Two feeding trials, one in winter and one in summer, were conducted with Holstein and Jersey calves to determine the comparative value of alfalfa and

prairie hays when fed to calves from birth or after 8 weeks of age. Measurements were made of growth, feed consumption and utilization, and levels of carotene and vitamin A in the blood plasma.

Calves fed alfalfa hay required less ($P < 0.01$) TDN per pound of gain than calves fed prairie hay, whether they were fed the hay from birth or after 8 weeks of age. A temporary advantage was obtained at 4 and 10 weeks in increased heart girth by the early feeding of either alfalfa or prairie hay.

Plasma carotene and vitamin A levels were unrelated to treatment or season, although carotene intake was higher for the calves fed alfalfa than for those fed prairie hay and was higher in the summer trial than in the winter trial.

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EFFECT OF SIRE AND SYSTEM OF MATING ON ESTIMATED EMBRYONIC LOSS[1]

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Embryonic death has in recent years been recognized as a major cause of lowered fertility in dairy cattle. The predisposing factors for embryonic mortality and their relative importance are as yet largely unknown. The purpose of this study was to determine the possible effects of sire and system of mating on embryonic mortality by 5 months of gestation.

MATERIALS AND METHODS

Data for this report were collected from the Holstein-Friesian herd at the Emmons Blaine, Jr., Experimental Farm, Lake Mills, Wisconsin. The data include the results of all services in the herd between January 1, 1950, and July 31, 1953, as well as the lengths of all estrual cycles during the same period. The herd is composed of six lines of breeding, with the following mating systems being followed in each line: outbred dams mated to outbred sires of another line to produce line-cross outbred calves (O-O matings), outbred dams mated to related sires to produce inbred calves (O-I matings), inbred dams mated to related sires to produce inbred calves (I-I matings), and inbred dams mated to inbred sires of another line to produce outbred offspring (I-O matings). In addition, outbred control animals for the herd are obtained by mating line-cross outbred dams to nonproject sires in various artificial breeding studs.

The inbred dams (59 in number) had a total of 91 service periods. Their coefficients of inbreeding ranged from 0.125 to 0.375; the mean was 0.233, with 41 of 59 coefficients between 0.20 and 0.30. The variation in inbreeding coefficients of the dams mated to a particular bull was not sufficient to permit classification within sire by degree of inbreeding.

Eighteen herd sires were used in the project during the period covered by this report. Only four of these project sires were considered to be used a sufficient number of times to justify classification of the results of their services by sire and system of mating; consequently, the services of all other project sires and nonproject sires were grouped together to allow classification by system

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of mating. This "other sires" mating group was the only one in which the I-O mating system was represented.

All services in the herd were made by artificial insemination as a precaution against the introduction and spread of genital infections. Semen from project sires was undiluted if used on the day of collection. If kept for use the following day, it was diluted 1:5 with Ortho diluent and stored at 4° C. No antibacterial agents were added. Processed semen in field use on the day of service was used in matings to nonproject sires. This semen was diluted with yolk–citrate or milk and contained sulfa and antibiotics.

RESULTS

The results of 837 services were included in the data. Of these, 404 (48.3%) resulted in pregnancies that were maintained to at least 150 days, 55 (6.6%) resulted in pregnancies that were lost before 150 days, and 378 (45.2%) were followed by return intervals varying in length from 5 to 99 days.

These return intervals did not include any cases in which animals returned to heat after chorionic membranes and fluid were palpated in the uterus at the routine pregnancy check 35-41 days after breeding, but in which no amniotic vesicle was present at that time. The fluid and membranes indicated that an embryo had begun to develop even though it degenerated before the pregnancy check; therefore, all such cases were included under pregnancies lost before 150 days.

Six of the 55 pregnancies which were lost by 150 days were observed abortions, 39 were apparently normal pregnancies at 35-41 days but were resorbed or lost unobserved before 150 days, and 10 instances were noted in which embryos had apparently degenerated by 35-41 days but chorionic membranes and fluid were still present in the uterus. Of the 449 apparently normal pregnancies at the time of diagnosis, 45 (10.0%) were lost before 150 days.

Comparison of variability in estrual cycle and return-interval lengths. Data were available on 1,200 estrual cycles for comparison with the return intervals. The modal length for both estrual cycles and return intervals was 21 days. The mean length for estrual cycles and return intervals was 24.11 days and 26.44 days, respectively. These means are considerably lower than the 30.8 and 34.9 days for noncopulatory and copulatory cycles reported by Chapman and Casida *(1)* for clinically normal dairy cattle, and the 30.6 and 35.7 days reported by Olds and Seath *(9)*. The lower means reported here are believed to be due to the closer observation for estrual behavior at the experimental farm than in most dairy herds, as well as the fact that early lost pregnancies which would not ordinarily be distinguished as such were eliminated from these return intervals.

The 1,200 estrual cycles and the 378 return intervals were grouped according to length and the two distributions compared by Chi square as a test of independence (Table 1). The effect of insemination was found to be a significant delay in return to heat ($P < 0.01$). A greater per cent of estrual cycles than return intervals was less than 22 days in length, whereas a greater per cent of

TABLE 1

A comparison of 1,200 estrual cycles with 378 return intervals following breeding

Length, days	Per cent of estrual cycles of a given length	Per cent of return intervals of a given length
<20	25.8	20.6
20	14.0	9.0
21	16.3	13.2
22	12.8	13.0
23-26	13.8	14.8
27-30	1.3	5.3
31-34	1.3	5.0
35-38	1.3	4.5
39-44	8.8	8.0
>44	4.6	6.6
	100.0	100.0

$X^2 = 66.18$ $P < 0.01$

return intervals than estrual cycles fell in each class interval from 22 through 38 days. Thus, only 42.8% of the return intervals was less than 22 days in length compared to 56.1% of the estrual cycles; 42.6% of the return intervals was in the 22-38 day range, as compared to 30.5% of the estrual cycles. Percentages of estrual cycles and return intervals of more than 38 days in length were approximately equal (13.4% and 14.6%, respectively). This would be expected, inasmuch as pregnancy examinations were on the average made by this time.

Estimation of embryonic mortality. Hammond *(5)* and Marion *et al. (8)*, using vasectomized bulls, found that the act of mating itself failed to lengthen the following cycle in dairy cattle; hence, there is no reason to believe that the act of artificial insemination would affect return-interval length. Since Laing *(7)* found degenerate embryonic membranes in the uterus 25 days after breeding and Tanabe and Casida *(15)* observed the same phenomena at 34 days, it was assumed that embryonic death affected the length of a considerable number of the return intervals in this study, thus causing a greater per cent of return intervals than estrual cycles to fall in the 22-38 day range. An estimate of the extent of such early embryonic mortality was obtained from the discrepancy between the per cent of estrual cycles and return intervals in this range. It was thus estimated that 28.4% of the 22-38 day return intervals fell in this range as the result of an embryo delaying the return to estrus. This figure is undoubtedly a conservative estimate of embryonic mortality, since it does not take into account embryos that died too soon to affect the length of return intervals.

All services were classified by mating group and system of mating, and the number of pregnancies at 150 days, number of pregnancies lost by 150 days, and number of return intervals 22 to 38 days in length were noted for each system-of-mating group (Table 2). An estimate of embryonic mortality to 150 days for each system of mating within mating group was then calculated by the following formula:

$$\% \text{ ED} = \frac{\text{Pr. lost by 150 d.} + 28.4\% \text{ of 22-38 d. RI}}{\text{Pr. at 150 d.} + \text{Pr. lost by 150 d.} + 28.4\% \text{ of 22-38 d. RI}} \times 100$$

TABLE 2
Estimated embryonic mortality by mating group and system of mating

Mating group	System of mating[a] Dams-Embryos	Number				Estimated embryonic mortality to 150 days
		Services	Pregnant at 150 days	Diagnosed pregnancy lost before 150 days	Return interval 22-38	
						(%)
O-A	O-O	57	19	4	16	31.0
	O-I	48	16	2	13	26.2
	I-I	50	8	5	17	55.1
	Group	155	43	11	46	35.9
20-B	O-O	39	19	1	10	16.8
	O-I	39	22	3	7	18.5
	I-I	13	6	2	3	32.2
	Group	91	47	6	20	19.9
40-A	O-O	48	28	3	6	14.4
	O-I	33	22	0	5	6.1
	I-I	13	8	1	0	11.1
	Group	94	58	4	11	10.9
80-A	O-O	45	21	4	5	20.5
	O-I	43	23	4	8	21.4
	I-I	16	7	1	5	25.7
	Group	104	51	9	18	21.7
Other Sires	O-O	170	104	12	21	14.7
	O-I	121	48	5	31	22.3
	I-I	28	14	1	3	11.7
	I-O	74	39	7	10	20.1
	Group	393	205	25	65	17.5
	Total	837	404	55	160	19.9

[a] O = outbred; I = inbred.

where *ED* is estimated embryonic death by 150 days, *Pr.* is number of pregnancies, and *RI* is number of return intervals.

Effect of mating group and system of mating on embryonic mortality. The over-all estimate of embryonic death by 5 months of gestation was 19.9%. Variation in the embryonic death rate was first analyzed for the effects of system of mating. The embryonic death rates for each system of mating within mating group (Table 2) were transformed to angles. All analyses were then made by analysis of Chi square by the method of Rao *(10)*, a method of analysis which takes into account unequal subclass numbers.

O-O matings (19.5% embryonic death when unweighted by mating group) were compared within mating group to O-I matings (18.9% embryonic death). The difference was not statistically significant (P between systems of mating $\cong 0.70$; interaction between mating group and system of mating $\cong 0.60$). O-O matings were then compared within mating group to I-I matings (27.2% embryonic death). The difference again was not statistically significant (P between systems of mating $\cong 0.35$; interaction between mating group and system of mating $\cong 0.60$). A comparison was also made within mating group between the

embryonic death rates of O-I and I-I matings (P between systems of mating $\cong 0.35$; interaction between mating group and system of mating $\cong 0.35$).

Since no statistically significant differences were found in embryonic death rates between any two of the three mating systems which were used in each mating group, the data in the four mating systems were combined within mating group in different ways in order to analyze further the variation in the embryonic death rate for effects of inbreeding of either the dam or the embryo. In testing for the effects of inbreeding of the embryo on the embryonic death rate, the embryos were classified by mating group and system of mating (outbred or inbred). Thus, O-O matings were compared to the combined within-mating group O-I and I-I matings. (I-O matings were combined with O-O matings in the "other sires" group.) Similarly, in testing for effects of inbreeding of the dam on embryonic mortality, the dams were classified by mating group and system of mating; O-O and O-I matings were combined within mating group and compared to the I-I matings. (I-O matings were combined with I-I matings in the "other sires" group.)

The outbred embryos had a death rate of 19.8% by 5 months, as compared to 22.3% for inbred embryos (Table 3). Outbred dams had 19.2% embryonic mortality by 5 months, whereas the incidence was 28.4% in the inbred dams. The embryonic death rates in females bred to the four heavily used project sires were 35.9, 19.9, 10.9, and 21.7% (Table 2). Other sires had an over-all embryonic death rate of 17.5%.

TABLE 3

Embryonic death rates of embryos and of dams when classified by mating group and system of mating

Mating group	Embryos		Dams	
	Outbred	Inbred	Outbred	Inbred
	(%)	*(%)*	*(%)*	*(%)*
O-A	31.0	39.3	28.9	55.1
20-B	16.8	21.9	17.7	32.2
40-A	14.4	7.5	10.9	11.1
80-A	20.5	22.5	21.0	25.7
Other sires	16.3	20.2	17.3	18.1
Mean[a]	19.8	22.3	19.2	28.4

[a] Unweighted mean of mating groups.

Outbred and inbred embryos did not differ significantly in mortality ($P \cong 0.50$). Neither did outbred and inbred dams differ significantly in embryonic mortality although the trend was stronger than for embryos ($P \cong 0.20$). Interactions between mating group and system of mating were not significant for either the embryos or the dams ($P \cong 0.80$ and 0.50, respectively), but the mating group was found to have a significant effect on embryonic mortality ($P < 0.01$ in each analysis).

Although the difference in embryonic mortality between inbreds and outbreds was nonsignificant, the trend for both inbred embryos and embryos in inbred dams, particularly the latter, to have a higher embryonic mortality rate than

the corresponding outbreds may be important. A further indication of this trend was noted in examination of the data for instances of more than one diagnosed pregnancy being lost in the same service period. Two pregnancies were lost in the same service period by each of two outbred females, but both were carrying an inbred embryo each time; three pregnancies were lost in the same service period by one inbred cow carrying an outbred embryo in each instance and by one inbred animal that was carrying an inbred embryo each time. In no case was more than one diagnosed pregnancy lost in the same service period by an outbred female carrying an outbred embryo.

DISCUSSION

The estimate of 19.9% embryonic death by 5 months of gestation is probably low for these bulls, since the method used in obtaining the estimate does not account for embryos that died too early to affect return-interval length. Kidder *et al. (6)* estimated embryonic mortality to 60-90 days as 21.0%, based on the discrepancy between 3-day fertilization rates and 60-90 day nonreturn rates of the same bulls, which were in field use with artificial breeding studs.

Fosgate and Smith *(4)* reported that 3.6% of 690 normal pregnancies diagnosed at 34-50 days were lost before 150 days; a comparable estimate from the present study is 10.0%, but diagnosis was made at 35-41 days.

The trend in the present study emphasizes the importance of work on an increased number of inbred dams and inbred embryos and also with an increased amount of inbreeding. There seem to be grounds for suspecting that inbreeding influences the embryonic death rate. Squiers *et al. (14)* found no significant differences in total embryonic mortality (fertilization failure and embryonic death to 25 days) among one outbred and three inbred strains of swine. However, they found that embryonic mortality to 25 days was reduced significantly in crossbred gilts from these four parental strains.

Several investigators have reported evidence suggesting that bulls have an effect on the embryonic death rate of cows to which they are bred. Rottensten *(11, 12)* found a lowered conception rate in return cows following service to low-fertility bulls and postulated that these bulls were carrying some "sterility-producing factor" or infectious agent that was causing embryonic death. Christian and Casida *(2)* reported that certain bulls used at first service affected the conception rate of other bulls used on their return cows at later services, and also noted that the lowered fertility produced in the cows by the first bull is much more apparent for low-fertility bulls than for average-fertility bulls. They found a definite association between the average length of the return interval and the fertility of the return cows at second service, long return intervals being associated with low fertility. Salisbury *et al. (13)* found significant variation among sires in the difference between 1-month and 5-month nonreturn rates, and also found a significant negative correlation between a bull's fertility and his apparent embryonic mortality. Flerchinger and Erb *(3)* concluded that low-fertility bulls lower the fertility of repeat service cows, even though repeat services are with semen from high-fertility bulls.

The studies cited above all suggest that low-fertility bulls have higher embryonic death rates than average or high-fertility bulls. Kidder *et al. (6)* found a group of bulls selected for their low nonreturn rates, if any different, actually to have a lower embryonic death rate than another group selected for their high nonreturn rates. These results, however, would not be contradictory to those cited above if selection against low-fertility bulls with high embryonic mortality was brought about some way in the artificial breeding studs prior to the time bulls were selected for the study.

Sire O-A had a low nonreturn rate throughout his period of service as a project sire, and an examination was made for possible disease factors that might have been the cause. Semen was collected as aseptically as possible from this bull during the tabulations for this report. It was both cultured and examined microscopically for the presence of *Vibrio fetus* organisms, but none were found. It was then decided to slaughter this bull, and swabbings of his genital organs were cultured for pathological bacteria. These cultures also failed to show the presence of such organisms.

SUMMARY

A total of 837 services were analyzed for the effects of sire and system of mating on estimated embryonic mortality by 150 days of gestation. The sire had a highly significant effect on the rate of embryonic death in the females to which he was bred. Outbred embryos had an estimated death rate of 19.8% by 150 days as compared to 22.3% for inbred embryos. Outbred dams had 19.2% embryonic death by 150 days, whereas embryonic mortality in inbred dams was 28.4%. Although the difference between outbreds and inbreds was not statistically significant in either case, the trend was for inbreeding of either embryos or dams, particularly the latter, to increase embryonic mortality.

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GENETIC ANALYSIS OF THE COMPONENTS OF TYPE CONFORMATION AND PRODUCTION IN AYRSHIRE COWS[1,2]

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The phenotypic and genetic correlations between final type score and production in dairy cattle have been estimated by Harvey and Lush *(3)* and by Touchberry *(7)*. Harvey and Lush's data show that for all practical purposes the genetic merit for over-all type conformation may be estimated independently of production and the genetic merit for production may be estimated independently of over-all type conformation with little loss in the accuracy of estimation of an individual's true breeding merit. In other words, when selection is practiced for production alone, little or nothing is to be gained by using type scores in addition to records of production. Touchberry estimated the phenotypic correlations between milk production and over-all type score and between butterfat production and over-all type score to be 0.182 and 0.258, respectively, and the genetic correlation was estimated to be zero in each instance. These estimates imply that type score should receive negative emphasis when utilized in the selection for butterfat production.

Since over-all type conformation is determined by the various components of type, it is possible that scores on certain of the components would be of value as additional information in the selection for over-all type conformation and/or production. Therefore, estimates of the phenotypic and genetic correlations between the components of type and final rating and the components of type and production are needed to determine how the components of type may be used in a selection program. It was the objective of this study to estimate the heritability of each of the components of type and final type rating—as currently defined by the Ayrshire Breeders' Association—as well as to estimate the phenotypic and genetic correlations between each component of type and final rating, between each component of type and butterfat production, between all components of type, and between final rating and butterfat production.

SOURCE OF DATA

The Ayrshire Breeders' Association, in a cooperative research agreement with the Dairy Department of West Virginia University, has made the breed association's records available for research purposes. On July 1, 1950, the association put into effect a modification of their type classification program that provided

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for the numerical scoring of ten components of type, which are averaged to determine the final type score. These components are: (1) head and neck, (2) shoulders and chest, (3) middle and loin, (4) rump and thighs, (5) feet and legs, (6) udder size and shape, (7) udder attachments, (8) udder teats, veins, and quality, (9) general quality, and (10) breed character. The maximum score given to any component is 100, and the minimum score, 50. All scoring was done by official classifiers of the association.

All daughter-dam comparisons available from records collected between July, 1950, and September, 1952, were used to estimate the various parameters. Single type and butterfat records were used in all instances and all type records were on cows which had freshened at least once. All butterfat records were made in the Herd Improvement Registry testing program and were converted to an M.E. 2 × 305 day basis by the breed association. An attempt was made to utilize concurrent production and classification records by selecting the production record with the most recent freshening date prior to the date of classification. Of the records selected for analysis, approximately 4.9% of the daughters' butterfat records were lactations which began after the date of classification and 2.0% of the dams' butterfat records were started after the date of classification.

ANALYTICAL PROCEDURE AND RESULTS

Since the data included only records made during a 27-month period, time trends were considered to be a negligible source of variation, and sire differences were believed to be of less importance than differences among herds. Harvey and Lush *(3)* found that differences among herds accounted for 18% and 44 to 48% of the total variation in type ratings and average butterfat production, respectively, of both the daughters and dams included in their Jersey data. For these reasons, and since 96.7% of the daughters and dams were in the same herd, the heritabilities and the phenotypic and genetic correlations were estimated on an intraherd basis.

Heritabilities. The average within-herd regression of daughter's trait on dam's trait was doubled to estimate heritability. The average number of daughters per dam in these data was 1.31, and when a dam had more than one daughter, the dam's record was repeated for each daughter's record. Kempthorne and Tandon *(6)* have recently presented a procedure for estimating heritability from a regression of offspring on parent which is superior to the procedure of using the dam's record repeatedly, in that their method provides estimates with minimum variance and the latter procedure does not. However, their procedure increases the complexity of computation somewhat without greatly enhancing the validity of the estimates of heritability.

Regression coefficients were estimated rather than correlation coefficients because Eisenhart *(2)* has shown that selection for the independent variable in a bivariate distribution does not introduce a bias in the estimate of the regression coefficient but does introduce a bias in the estimate of the correlation coefficient. In this instance, however, the means and intraherd standard deviations for

TABLE 1
Mean and intraherd standard deviation of daughters' and dams' final type rating and butterfat production

	Daughters		Dams	
	Mean	Standard deviation	Mean	Standard deviation
Final type rating	82.9	3.0	83.2	3.2
Butterfat production	418.2	77.3	418.4	84.7

daughters' and dams' final ratings and production records presented in Table 1 indicate that selection of the dams had been no greater than the selection of the daughters.

The number of daughter-dam comparisons used to estimate the heritabilities of the various components of type and final rating differed because of the deletion of impossible scores for one or more components. Under the rules of the classification program, scores less than 50 or greater than 100 are impossible by definition. The number of daughter-dam pairs, the estimate of heritability for each component of type and final rating, and the respective 95% confidence limits are presented in Table 2.

The estimates of heritability of head and neck, middle and loin, rump and thighs, udder teats, veins and quality, breed character, and final rating are of approximately the same magnitude, being of the order of 0.30. The estimates for the three components of udder conformation differ markedly, udder attachments and udder size and shape being essentially zero, whereas the estimate for teats, veins, and quality is 0.27. The estimate of 0.31 for the heritability of final rating agrees closely with the estimate of 0.30 obtained from similar data by Tyler and Hyatt *(8)*. The heritability of final rating has also been estimated by Touchberry *(7)* to be 0.00 and by Harvey and Lush *(3)* to be 0.14.

Phenotypic correlations. The phenotypic correlations between the components of type, final rating, and butterfat production were estimated to measure the degree of association of these traits in the same animal. Single records on 1,273 cows located in 170 herds were used to make these estimates, which are presented in Table 3.

TABLE 2
Heritabilities of the components of type and final type rating with their respective 95% confidence limits

Trait	No. of pairs	Heritability	95% confidence limits
Head and neck	1190	0.30	±0.12
Shoulders and chest	1180	0.15	±0.14
Middle and loin	1182	0.31	±0.18
Rump and thighs	1182	0.32	±0.16
Feet and legs	1173	0.18	±0.15
Udder size and shape	1176	0.08	±0.14
Udder attachments	1175	0.06	±0.11
Udder teats, veins, and quality	1182	0.27	±0.16
General quality	1182	0.13	±0.15
Breed character	973	0.32	±0.16
Final rating	1184	0.31	±0.15

TABLE 3

Phenotypic correlations between the components of type, final type rating, and butterfat production

Trait	Shoulders and chest	Middle and loin	Rump and thighs	Feet and legs	Udder size and shape	Udder attach-ments	Udder teats, veins, and quality	General quality	Breed character	Final rating	Butterfat production
Head and neck	0.28	0.31	0.22	0.15	0.20	0.55	0.26	0.43	0.48	0.50	0.05
Shoulders and chest		0.26	0.18	0.17	0.16	0.15	0.20	0.43	0.42	0.51	0.02
Middle and loin			0.47	0.14	0.22	0.22	0.22	0.38	0.55	0.57	0.08
Rump and thighs				0.18	0.19	0.23	0.22	0.44	0.60	0.60	0.00
Feet and legs					0.14	0.16	0.12	0.31	0.42	0.50	0.00
Udder size and shape						0.50	0.78	0.36	0.52	0.66	0.10
Udder attachments							0.45	0.30	0.39	0.58	0.04
Udder teats, veins, and quality								0.44	0.54	0.67	0.12
General quality									0.59	0.72	0.14
Breed character										0.87	0.07
Final rating											0.08

TABLE 4

Genetic correlations between the components of type, final type rating, and butterfat production

Trait	Shoulders and chest	Middle and loin	Rump and thighs	Feet and legs	Udder size and shape	Udder attach-ments	Udder teats, veins, and quality	General quality	Breed character	Final rating	Butterfat production
Head and neck	0.09	0.29	0.30	0.18	0.48	1.03	0.27	0.89	0.64	0.64	–0.51
Shoulders and chest		–0.17	0.45	0.42	0.83	0.51	0.38	0.61	0.31	0.60	–0.60
Middle and loin			0.86	0.51	0.87	0.36	0.47	0.90	0.75	0.73	0.22
Rump and thighs				0.61	0.32	–0.15	0.02	0.68	0.87	0.65	–0.40
Feet and legs					0.18	0.27	–0.18	0.41	0.41	0.55	–0.09
Udder size and shape						1.19	1.20	6.43	1.04	1.07	–0.64
Udder attachments							1.10	1.13	0.59	0.93	–0.25
Udder teats, veins, and quality								0.53	0.64	0.64	–0.39
General quality									1.05	1.00	–0.86
Breed character										0.91	–0.50
Final rating											–0.52

In general, the phenotypic correlations between butterfat production and all components and final rating were relatively low, the highest being 0.14 between general quality and butterfat production. Relatively high correlations existed between all components of type and final rating, as would be expected, because final rating is an average of the component scores. The correlations between the various components are quite variable, ranging from 0.12 between feet and legs and udder teats, veins, and quality to 0.78 between udder size and shape and udder teats, veins, and quality.

The estimate of 0.08 for the phenotypic correlation between final rating and butterfat production is lower than the corresponding estimates reported by other workers. Touchberry *(7)* estimated this correlation from 367 records to be 0.26 on an intrasire basis; Tyler and Hyatt *(8)* utilized first lactation records on 5,177 cows and obtained a within-herd estimate of 0.16. Their estimate was subject to the least sampling error because of the large sample size. In contrast to the intrasire and intraherd estimates, Copeland *(1)* estimated the gross phenotypic correlation between final rating and butterfat production from the highest records on 695 Jersey cows to be 0.25.

The highest phenotypic correlation found between any individual component and final rating was the correlation between breed character and final rating; the next highest was between general quality and final rating. Perhaps these high correlations could be anticipated, inasmuch as breed character and general quality are somewhat dependent on over-all conformation of the cow rather than on a particular region, such as the rump and thighs.

Genetic correlations. The estimation of genetic correlations has been described by Hazel *(4)* and applied to dairy cattle problems by Harvey and Lush *(3)* and by Touchberry *(7)*. Genetic correlations were estimated from these data by the same procedure except that the appropriate crossproducts were used rather than regression or correlation coefficients.[4] If X_i denotes the i^{th} trait on a daughter and Z_j the j^{th} trait on her dam, then the genetic correlation between the i^{th} and j^{th} trait is:

$$(1)\quad r_{g_i g_j} = \frac{\sigma g_i g_j}{\sqrt{\sigma^2 g_i \ \sigma^2 g_j}} = \frac{\frac{1}{2}\left(\Sigma X_i Z_j + \Sigma X_j Z_i\right)}{\sqrt{\left(\Sigma X_i Z_i\right)\ \left(\Sigma X_j Z_j\right)}}$$

The crossproducts estimate the following variances and covariances:

$$E\left\{\frac{1}{n-1}\left[\Sigma X_i Z_i\right]\right\} = \sigma X_i Z_i = \tfrac{1}{2}\ \sigma^2 g_i$$

$$E\left\{\frac{1}{n-1}\left[\Sigma X_j Z_j\right]\right\} = \sigma X_j Z_j = \tfrac{1}{2}\ \sigma^2 g_j$$

$$E\left\{\frac{1}{n-1}\left[\Sigma X_i Z_j\right]\right\} = \sigma X_i Z_j = \tfrac{1}{2}\ \sigma g_i g_j$$

$$E\left\{\frac{1}{n-1}\left[\Sigma X_j Z_i\right]\right\} = \sigma X_j Z_i = \tfrac{1}{2}\ \sigma g_i g_j$$

[4] This procedure was suggested to the authors by Dr. C. R. Henderson.

where E represents the expected value of a crossproduct, $\sigma^2 g_i$ is the additive genetic variance of the i^{th} trait, and $\sigma g_i g_j$ is the additive genetic covariance between the i^{th} and j^{th} trait. This method assumes that the dams were not selected, which seems to be true or nearly so in the case of these data. If selection has been effective in the population, the geometric mean of the two covariances in the numerator of Equation 1 would be appropriate because it would yield an unbiased estimate of the genetic correlation under such a condition, whereas the arithmetic mean would not.

A total of 729 daughter-dam comparisons located in 165 herds was used to estimate the genetic correlations presented in Table 4. The estimates of the genetic correlations between the components of type and butterfat production were all negative with the exception of the correlation between middle and loin and butterfat production. The genetic correlations between the components of type and between the components of type and final rating varied from –0.18 to greater than 1.00. Although it is not possible for a correlation to be greater than 1.00 or less than –1.00, it is possible for an estimate of a correlation of this type to be greater than unity because of sampling errors.

The estimate of the genetic correlation between final rating and butterfat production obtained from these data is –0.52. This is lower than the estimate of zero obtained by Touchberry from 187 Holstein daughter-dam pairs and the estimate of 0.18 obtained by Harvey and Lush from 2,786 Jersey daughter-dam comparisons.

The relative magnitudes of the phenotypic and genetic correlations vary considerably from one pair of traits to another. For example, the greatest phenotypic correlation between any trait and butterfat production is the correlation between general quality and butterfat production, whereas the corresponding genetic correlation between these two traits is the lowest value found. This is a reflection of the fact that phenotypic correlations are a consequence of genetic and environmental components. In general quality and butterfat production the difference between the phenotypic correlation of 0.14 and the genetic correlation of –0.86 indicates that the effect of an environmental correlation is very pronounced. Such considerations make it obvious that phenotypic correlations are of limited value in selection unless something is known of the relative magnitudes of their environmental and genetic components.

DISCUSSION

Heritability is often defined as the ratio of additive genetic variance to total or phenotypic variance. The estimates of heritability obtained from these data would not be biased by dominance deviations since they are based on daughter-dam comparisons, but they would include a fraction of the epistatic effects. Similarly, the genetic correlations would be unaffected by dominance deviations but would include additive genetic variance plus a small part of the epistatic variance.

Since certain genetic correlations were estimated from these data to be posi-

tive and others to be negative, it may be well to point out the implications of such estimates. If a genetic correlation between two traits is negative, then dams that are better than average with respect to one trait would be expected to have daughters that are poorer than average with respect to the second trait. Similarly, dams which are poorer than average in Trait 1 would be expected to have daughters which are better than average in Trait 2. If the genetic correlation between two traits is positive, then dams that are better than average with respect to one trait tend to have daughters that are better than average in Trait 2, and similarly, dams which are poorer than average with respect to Trait 1 tend to have daughters which are poorer than average with respect to Trait 2.

A large part of the variation due to differences among inspectors has probably been eliminated from these estimates because 96.7% of the daughters and dams were in the same herd and, because the data were collected during a 27-month period, were probably classified at the same time. Although inspector differences were largely eliminated from these estimates, it is questionable from a practical point of view whether or not such estimates are the most desirable. A breeder would ordinarily have to work with scores made by different inspectors, and the estimates of heritability and correlations which contain inspector differences would seem to be more readily applicable.

In practice many commercial dairymen are interested in selecting for milk or butterfat production alone. The relative emphasis that final type rating and production should receive when a breeder is interested in selecting for production alone can be determined by using a linear index:

$$(2) \quad I_{12} = b_{11}X_{11} + b_{12}X_{12}$$

where X_{11} and X_{12} represent a final rating and a butterfat production record, respectively. The b's are multiple regression coefficients which determine the weight that should be given final rating and butterfat production. The b's are determined by solving the following set of equations:

$$(3) \quad \begin{aligned} b_{11}\sigma^2X_{11} + b_{12}\sigma X_{11}X_{12} &= \sigma g_{11}g_{12} \\ b_{11}\sigma X_{11}X_{12} + b_{12}\sigma^2X_{12} &= \sigma^2 g_{12} \end{aligned}$$

where

σ^2X_{11} = phenotypic variance of final type rating
σ^2X_{12} = phenotypic variance of butterfat production
$\sigma X_{11}X_{12}$ = phenotypic covariance between final type rating and butterfat production
$\sigma g_{11}g_{12}$ = additive genetic covariance between final type rating and butterfat production
$\sigma^2 g_{12}$ = additive genetic variance of butterfat production

By substituting in Equations 3 the appropriate sums of squares and cross-products computed in the estimation of the phenotypic and genetic correlations, the following b values were determined: $b_{11} = -0.99$ and $b_{12} = 0.04$. These values show that nothing can be gained by giving positive weight to final type rating when one is interested in selecting for butterfat production alone. In fact, if these estimates are taken at face value, final rating should receive negative weight

in the index. The correlation (r_{TI}) between the true breeding value for butterfat production T and the index I which estimates T is 0.28. An estimate of the heritability of butterfat production obtained from the genetic correlation data was 0.06 with an upper 95% confidence limit of 0.21. Based on this estimate of heritability, r_{TI} is 0.24 when selection is carried out with only a single butterfat record. In a similar manner an appropriate index for a breeder interested in selecting for final rating alone is:

$$(4)\quad I_{11} = 0.15X_{11} - 0.002X_{12}$$

In this instance little or nothing is gained by giving consideration to butterfat records.

The weights that should be given each component of type when selecting for butterfat production can be estimated by the same procedure (6). The index would be:

$$(5)\quad I_{12} = b_1X_1 + b_2X_2 +, \ldots\ldots\ldots, + b_{10}X_{10} + b_{12}X_{12}$$

The weight which should be given to each component and final type rating (X_{11}) when selecting for butterfat production cannot be estimated simultaneously from these data because final type rating is an average of the ten component scores.

The b values are determined by solving the following set of equations:

$$(6)\quad \begin{array}{llllll} b_1\sigma^2X_1 & + b_2\sigma X_1X_2 & + \text{———} & + b_{12}\sigma X_1X_{12} & = & \sigma g_1g_{12} \\ b_1\sigma X_1X_2 & + b_2\sigma^2X_2 & + \text{———} & + b_{12}\sigma X_2X_{12} & = & \sigma g_2g_{12} \\ \vdots & \vdots & & \vdots & & \vdots \\ b_1\sigma X_1X_{12} & + b_2\sigma X_2X_{12} & + \text{———} & + b_{12}\sigma^2X_{12} & = & \sigma^2g_{12} \end{array}$$

The left-hand members of these equations are phenotypic variances and covariances, and the right-hand members are additive genetic covariances and the additive genetic variance of butterfat production. These equations are readily solved with a desk calculator. The b values or weights that should be given to each component of type and a single butterfat record on an individual when selecting for butterfat production are estimated from these data to be as follows:

b_1 — head and neck: –1.03
b_2 — shoulders and chest: –0.25
b_3 — middle and loin: 1.20
b_4 — rump and thighs: –0.33
b_5 — feet and legs: –0.18
b_6 — udder size and shape: –0.54
b_7 — udder attachments: 0.52
b_8 — udder teats, veins, and quality: 0.16
b_9 — general quality: –0.72
b_{10} — breed character: –0.12
b_{12} — single butterfat record: 0.04

These weighting factors or regression coefficients are applicable only when all of the component scores, plus a butterfat record, are to be used in selecting

for butterfat production. If one should wish to use a certain few rather than all of the components, it would be necessary to derive the appropriate coefficients by solving the necessary equations.

For this index, Equation 5, r_{TI} is 0.46. This increase in r_{TI} from 0.28 to 0.46 might have been expected, inasmuch as r_{TI} is a multiple correlation coefficient, and the inclusion of the components of type in the index would take into account more variation than would the use of final rating, which is an average of the components. In actuality, if the two indexes were applied to an independent set of data, the index which utilizes the component scores and a production record might not be as superior to the index using final rating and production as the difference in the values of r_{TI} for the two indexes indicates. This situation could be brought about by sampling errors associated with the estimates of the variances and covariances used in computing the indexes. The estimate of heritability of butterfat production obtained from these data was lower than might have been expected and, as a result, the values of r_{TI} were also rather low.

Perhaps the most striking finding of this work is that the highest value of r_{TI} was obtained by giving negative emphasis to seven of the ten components of type, but the weights that have been presented in these indexes should be considered as only approximate because it appears that these data do not include enough observations from which to estimate the genetic relationship between the components of type and butterfat production as accurately as would be desired. However, these data at least serve to illustrate a procedure by which these weights may be determined.

SUMMARY

The heritabilities of ten components of type and final rating were estimated from approximately 1,180 daughter-dam comparisons by doubling the intraherd regression of daughter's single type score on dam's single type score. The heritability estimates are as follows: head and neck, 0.30; shoulders and chest, 0.15; middle and loin, 0.31; rump and thighs, 0.32; feet and legs, 0.18; udder size and shape, 0.08; udder attachments, 0.06; udder teats, veins and quality, 0.27; general quality, 0.13; breed character, 0.32; and final rating, 0.31.

Single type and butterfat records were used to estimate the average within-herd phenotypic and genetic correlations between all combinations of the components of type, final type rating, and butterfat production. Records on 1,273 cows were used to estimate phenotypic correlations, and 729 daughter-dam pairs were used to estimate genetic correlations. The phenotypic correlation between final type rating and butterfat production was estimated to be 0.08, and the corresponding genetic correlation was estimated to be –0.52. From these rather limited data and because of the sampling error involved, the estimates of genetic correlations can only be considered as approximate. Even so, it seems that the genetic correlations between butterfat production and final type rating and between butterfat production and the components of type are low at best.

The weights which should be given to the records on each component of type and a single butterfat record on an animal when selecting for butterfat produc-

tion were determined. These data indicate that in selecting for butterfat production alone nothing can be gained by giving positive emphasis to final type score.

ACKNOWLEDGMENTS

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TECHNICAL NOTES

ON THE MECHANISM OF ACTIVATION OF LIPOLYSIS AND THE STABILITY OF LIPASE SYSTEMS OF NORMAL MILK[1]

An understanding of the mechanism of activation of induced lipolysis in milk is essential for the effective control of rancidity. A distinction has been made between "induced" lipolysis, which involves the lipase system of normal milk, and "spontaneous" lipolysis, which occurs upon the cooling of naturally lipolytically active milk produced by certain cows *(3)*. The activation treatments known to affect normal raw milk include homogenization, prolonged agitation of warm milk, and "temperature activation," i.e., warming precooled milk to 30° C. and cooling again to below 10° C. It has been generally considered that these treatments involve changes in the state of the substrate, making it available to lipase action.

Because of a high incidence of lipolysis on dairy farms where pipeline milkers have been adopted, a study was undertaken to obtain an understanding of the mechanism of activation of lipolysis by "air agitation." In a discussion of this problem, Herrington *(1)* commented: "It is difficult to understand why agitation of warm raw milk with air in a vertical pipe should produce much more activation than agitation for the same time in a Waring Blendor. That is the case if the Waring Blendor is filled completely so that no space is left for air."

"Foam Theory" of activation by air agitation. We have sufficient evidence at present to show that the formation of foam is an important feature of the mechanism involved in the activation of lipolysis by agitation with air (as it occurs in pipeline milkers). The necessary conditions for activation by "air agitation" appear to be foaming, with continuous mixing of foam and milk at temperatures that keep the milk fat in a liquid state. These conditions seem to be ideally provided in pipeline milkers if risers, air leaks, and certain types of filters are present in the system. In Herrington's experiment, the lack of air space in the Waring Blendor prevented foam formation.

The importance of foam formation for this type of activation is indicated by the following observations:

1. Drawing air through a fine-fritted glass disk in a column of milk at 37° C. at a rate to produce efficient foaming effected the activation of lipolysis, although agitation was very mild. Gentle mixing of the foam with the rest of the liquid and maintaining the temperature of milk and foam at 37° C. are the only conditions necessary for maximum activation (Ex-

[1] This study was supported by funds from the California Dairy Industry Advisory Board.

TABLE 1

Activation of lipolysis in raw milk by foaming and shaking at 37° C.

Description of samples	F.F.A.[a] after 24 hr. storage at 4° C.
Experiment 1	
Control, not treated	2.56
Foamed, 10 min.	3.78
Foamed, 10 min. with interruptions and mixing every 5 min.	4.15
Foamed, 30 min.	5.37
Foamed, 30 min. with interruptions and mixing every 5 min.	10.22
Experiment 2	
Control, without antifoaming agent	2.28
Control, with antifoaming agent[b]	2.43
Foamed, 2 min. without antifoaming agent	6.87
Foamed, 12 min. with antifoaming agent	3.44
Shaken, 30 min. without antifoaming agent	7.56
Shaken, 30 min. with antifoaming agent	2.52
Experiment 3	
Control, raw milk	2.48
Control, past. milk	2.67
Control, 1 vol. raw milk + 1 vol. past. milk	2.61
Pasteurized milk, shaken	2.37
Raw milk, shaken	13.6
1 vol. raw milk, not shaken + 1 vol. past. milk, shaken	4.26
1 vol. raw milk, shaken + 1 vol. past. milk, not shaken	9.70

[a] Free fat acidity: ml. 1 N alkali required to neutralize ether extract from milk containing 100 g. fat, as determined by an extraction-titration method. (*J. Dairy Sci.*, 37: 646. 1954).

[b] Dow Corning antifoam AF emulsion, added at the rate of 70 mg. per 40 ml. milk.

periment 1, Table 1). When air was drawn or bubbled through milk at such rate that no foaming took place, partial churning occurred in a relatively short time. Samples withdrawn at intervals during treatment, before and after significant churning took place, showed no activation of lipolysis.

2. The addition of an antifoaming agent (Dow Corning antifoaming AF emulsion[2]) considerably diminished—or prevented entirely—the activation of lipolysis by means of foaming or vigorous shaking in a Warburg apparatus (Experiment 2, Table 1). This antifoaming agent had no diminishing effect on lipolytic activity when it was added to milk after activation had been induced by shaking. The addition of rancid milk to fresh milk at 5 and 10% levels before shaking or foaming treatments also reduced the extent of activation. In the foaming treatment the fatty acids acted as effective antifoaming agents.

3. Foaming or shaking milk with nitrogen or oxygen had the same effect as air on induced lipolysis. Therefore the role of oxygen in air is the same as that of any gas in forming foam.

It is significant to note that the extent of oiling-off or partial churning was essentially the same in the presence or absence of foam, though sometimes more pronounced in the latter case. It is therefore apparent that the disruption of the natural membrane material surrounding fat globules in milk, making the substrate available to the enzyme, is not the only factor involved in the mechanism of activation.

The data of Experiment 3 (Table 1) show that considerably more activation of lipolysis took place in shaken milk when the enzyme source was active (raw milk) than when pasteurized milk was shaken separately and then mixed with an equivalent amount of raw milk. The same results were obtained when the activation was carried out by a combination of foaming and shaking (30 minutes each). It appears therefore that the activation by agitation is dependent for its effectiveness not only upon changes in the substrate, as has been recently emphasized *(1)*, but also upon effects on the enzyme itself. A similar experiment carried out on activation by homogenization confirms previous reports *(2)* that this type of activation seemingly does not affect the enzyme. On the other hand, the addition of antifoaming agent (Dow's AF) to raw milk before homogenization did not affect the extent of activation of lipolysis. This would indicate that different mechanisms are involved in activation by agitation and activation by homogenization.

Lipase is a surface reaction. Foam promotes the activation of lipolysis by providing optimum conditions, as follows: (a) greatly increased liquid surface; (b) selective concentration of enzyme at the surface (air-liquid interface); (c) activation of the substrate by surface denaturation of the membrane materials surrounding fat globules; and (d) intimate contact of enzyme and activated substrate. It is logical to assume that other treatments providing these conditions would be effective in activating lipolysis. Antifoaming agents or fatty acids, by virtue of their greater surface activity, are preferentially adsorbed at the air-liquid interface of the foam and prevent selective concentration of enzyme at this surface.

The data presented in this paper suggest that lipase of milk is not readily susceptible to denaturation by foaming. It appears therefore that foaming may be used as a means of concentration and isolation of lipase. This is being currently investigated in this laboratory.

It is evident that through proper design and use the conditions in pipeline milkers that promote increased surface by foaming or otherwise should be eliminated or brought to a minimum. These factors rather than agitation per se must be controlled to prevent activation of lipolysis by air agitation.

Stability of lipase in milk. In the course of a study of the lability of lipase systems in milk it was found that storing 24 hours at 0-4° C. before activation by homogenization or "agitation" decreased the extent of lipolysis 20 to 50% below that of the same milk activated without aging. In the case of "temperature activation" the lipolytic activity decreased 80 to 90% after the same length of storage in cold. This is further evidence for the complexity of lipase systems in milk *(3)*. The prior history of raw milk samples must be taken into consideration in investigations of induced lipolysis.

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[2] Obtained from the Dow Corning Corp., Midland, Mich.

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HEREDITARY EDEMA IN AYRSHIRE CATTLE[1]

After studying 325 cases of edema (dropsy or anasarca) in Ayrshire calves, Donald *et al. (1)* of Scotland concluded that the disease is hereditary. They found a preponderance of males affected but concluded that a less severe condition in females may not be recognized. A similar, if not identical, condition has been observed by Korkman *(4)* in Ayrshire calves in Finland. Evidence is reasonably conclusive that the condition is inherited as a single autosomal recessive.

This type of congenital edema is believed to be rare on the North American continent. In Ayrshires at the University of British Columbia 10 cases have been recorded *(3)*. Eight of these were sired by a bull that was a half-sib of two edematous calves. The case being discussed in this report is one of two animals previously reported by Eldridge and Atkeson *(2)*, the only cases reported to date in the United States. The original report was made in order to call attention of breeders to the hereditary nature and economic aspects of the condition. The additional observations made upon dissection of this animal seem worthy of making a matter of record.

FIG. 1. Edematous animal (left) at 3 months of age compared with normal Ayrshire calf of the same age.

FIG. 2. Front foot of edematous animal.

FIG. 3. Edema of the eye showing ballooning of the conjunctival membrane at 3 months of age.

The Ayrshire bull calf studied was born from registered parents that had produced an edematous calf at a previous calving. It showed extensive edema from birth, especially evident in extremities and head, the eyes being markedly affected (Figures 1, 2, and 3). All observations indicated this calf to be typical of the calves reported by Donald *et al.*

[1] Contribution No. 256, series 301, Department of Zoology and contribution No. 229 Department of Dairy Husbandry, Kansas Agricultural Experiment Station, Manhattan.

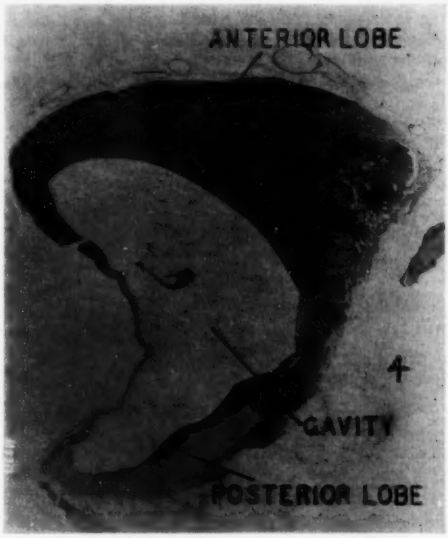

FIG. 4. Median longitudinal section of pituitary gland from edematous animal.

The calf was brought to the college and kept until it was 16 months of age and weighed 815 lb. Then it was killed and preserved with formalin intravascularly for use as a laboratory demonstration specimen for anatomy classes.[2] As the animal was progressively dissected, the organs and tissues were examined. Dermal and subcutaneous tissues were found to be infiltrated with fluid in addition to unusual development of fibrous tissues so that in places the skin was 1 in. or more in thickness.

A semen sample was obtained from this animal at 13 months of age by means of an artificial vagina. No females were inseminated, but microscopical examination revealed a sufficient number of progressively motile spermatozoa to indicate that the animal was not sterile. The apparent fertility of this male is evidence not only that the heterozygous carrier can transmit the causative gene but also that the homozygous individuals may be able to reproduce. Since this characteristic is variable in expression, as pointed out by Donald *et al. (1)*, a slightly affected bull used for breeding purposes could spread the condition widely.

One of the most striking abnormalities was found in the pituitary gland. A cyst containing fluid of undetermined origin and content replaced a large portion of the gland. A median

[2] Dissected by Wm. M. McLeod, Head of Department of Anatomy, School of Veterinary Medicine, Kansas State College.

FIG. 5. Median longitudinal section of pituitary gland from normal animal.

longitudinal section (Figure 4) and sections from other regions of the gland revealed a small amount of posterior pituitary tissue compared with the normal gland (Figure 5). The tissue of the posterior lobe appeared normal microscopically. The anterior lobe, likewise, was deficient quantitatively.[3] In a microscopical study of the anterior lobe, no striking abnormality was found, but there were fewer chromophobic cells than usual compared with the number of actively secreting cells. Well-defined acidophilic and basophilic cells were present, but extensive counts of these types of cells were not made.

The cyst in the pituitary gland and the edema were the only abnormalities found on dissection. Blood examination from the living animal gave a total leucocyte count of 10,375 and an erythrocyte count of 6,870,000 per cubic millimeter of blood and a hemoglobin value of 14.0 g. per 100 ml. of blood.[4] All of these values are well within the normal range for cattle.

In so far as is known, the pituitary glands of the 325 edematous calves of the Donald *et al.* series have not been studied. It may be merely coincidental that the animal reported here had edema and at the same time possessed an abnormal pituitary gland. It is believed, however, that this fact is worthy of report so that if further cases are observed, the pituitary gland may be examined to establish whether it commonly is abnormal in calves affected with congenital edema.

Observation of the one animal indicates that this hereditary trait is present in American herds. According to Donald *et al.*, losses from this condition in other countries have not been serious from the standpoint of the total numbers within the breed, but for the individual breeder the loss may be severe, since the dam may die at parturition.

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Departments of Zoology and Dairy Husbandry
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[3] Tissue sections prepared by Alice Kimball, Department of Pathology, School of Veterinary Medicine, Kansas State College.

[4] Determined by Department of Physiology, School of Veterinary Medicine, Kansas State College.

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PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

EDWARD SEWALL GUTHRIE was born in Coin, Iowa, Dec. 27, 1880, the eldest of a family of five boys. His boyhood was spent in rural Iowa, and he was educated in the public schools with the addition of a preparatory course at Amity College at College Springs, Iowa. His undergraduate training was obtained at the Iowa State College, from which he was graduated in 1905.

E. S. Guthrie

That Dr. Guthrie showed early promise of the fruitful career he was to follow is shown by the fact that, while he was still a senior at Iowa State, he was invited by Professor JOHN W. DECKER of The Ohio State University to teach a course in butter-making to winter-course students. After his graduation he obtained practical experience by working in dairy plants and then became an instructor in dairying at The Ohio State University, where he remained until 1908.

Dr. Guthrie's career at Cornell University started in the fall of 1908 when he became an instructor and began pursuing his graduate studies. He was awarded an M.S. degree in 1910 and a Ph.D. degree in 1913. During this period of graduate study he was also carrying a large share of the duties of the department. The value of his services and the outstanding promise he showed were appreciated by the University and he was early promoted to a full professorship of dairy industry, which position he held for 35 years before being retired on July 1, 1948.

Doctor Guthrie's many and varied contributions to dairy science are well known. He has been the author or co-author of more than 50 scientific publications, in addition to many articles for trade journals and similar publications. His book, *The Book of Butter*, published in 1918 and revised in 1923, was long a standard text in its field.

Although Dr. Guthrie was associated with many types of studies in dairy science, his first scientific love was the subject of butter, in all of its technological, economic, and practical phases. His early investigations in butter were those that dealt with the flavor changes, such as rancidity and the so-called metallic flavor; he was among the early investigators to recognize the fact that some of the abnormal and objectionable flavors of milk were caused by chemical changes that took place on storage. The latter part of his research career has been largely concerned with milk flavors, a subject on which he has been a well-known authority for years through his scientific and practical contributions and his expertness as a judge.

In his teaching career, Dr. Guthrie's principal courses were those having to do with butter, general dairying, and the grading of dairy products. He coached the Cornell Dairy Products Judging Team for 15 years and was one of the founders of the Cornell Dairy Science Assoc. for students.

Professor Guthrie was a charter member of the organization founded at Urbana, Ill., in 1906, that is now the American Dairy Science Assoc. The first name of our Association was the "National Assoc. of Dairy Instructors and Investigators;" at the second meeting, held in Chicago in 1907, the name was changed to "The Official Dairy Instructors Assoc." In 1917, the same year in which the *Journal of Dairy Science* was founded, the present name was officially adopted. Of the group of charter members, four survive—E. S. GUTHRIE, O. F. HUNZIKER, CHARLES THOM, and C. C. HAYDEN.

Professor Guthrie has been a very active member of our Association continuously since its founding and has been held in high esteem by his associates. He has served in many capacities, including the chairmanship of the general program committee and of the Eastern Section and membership in innumerable committees. He was president of the Association in 1941. In 1944 Professor Guthrie was elected to Honorary Life Membership in A.D.S.A.

In 1907 Dr. Guthrie was married to Margaret Fay Watterson of Beaver, Pa. They have four children and nine grandchildren.

Dr. Guthrie has few equals in the amount of time he has spent in the religious, charitable, and civic activities of the city and county. He has been active in the Forest Home Chapel from its early years, first as treasurer, then as a trustee, and as a steward to the present time. For 26 years he was superintendent of the Church School. For 30 years he has been active and held offices in the Tompkins County Council of Churches. Since 1932 he has been a member of the board of directors of the Reconstruction Home for Infantile Paralysis, and for the last

14 years he has conducted the Protestant services every Sunday morning in that home.

No statement about Doctor Guthrie, however short, could neglect his activities since his "retirement." He has continued to be just as active as in former years in his research work and his labors for the department. He comes to work regularly, pursuing his researches on milk flavors and rendering many other services to the department. One of his hobbies has been the writing of an annual letter to the previous students of the department, keeping them informed about the department affairs and furnishing them with the addresses of former students.

All those who have been associated with Dr. Guthrie hold him in the highest respect and the greatest affection. To know him is to love him, and there is no doubt that he has been revered and loved by as many students and associates over as great a number of years as has any other person who has ever been associated with Cornell's Department of Dairy Industry.

Dr. Guthrie richly deserves to be listed among the pioneers in the American dairy industry.

J. M. SHERMAN

L. F. Nafis Dies

The dairy industry has lost one of its pioneers and staunch supporters in the death at Evanston, Ill., on Feb. 26 of LOUIS FIRTH NAFIS, founder and manager of Louis F. Nafis, Inc., Scientific Dairy Laboratory Glassware. He was born Nov. 1, 1874, in Jersey City, N. J. Surviving are his wife, Mabel, and a sister, Miss Emily Nafis. Until his retirement, Mr. Nafis was a familiar figure at the national dairy expositions, where he always displayed his company's latest developments. He was an active member in the A.D.S.A. even after retirement and mentioned to his wife shortly before his death that he wished to keep up his membership in the Association as long as he lived. He was also active in the Masonic lodge.

Louis Nafis started his career selling thermometers. Some of his friends in the butter industry later got him interested in supplying them with accurate test bottles and pipettes. This assignment took him to the Univ. of Wisconsin, where he conferred with Professors Babcock and Farrington. He later referred to them as the godfathers of his business, for through their help he started a company to manufacture accurate glassware for the dairy laboratory. In 1932 he sold his business to the Kimble Glass Co., which was later taken over by Owens-Illinois Glass Co.

Michigan Awaits You

Members of the A.D.S.A. attending the June meetings at Michigan State College will have an opportunity to observe an industry that produces the largest income of any Michigan farm enterprise. From a humble beginning in 1701 at Fort Pontchartrain, now Detroit, has risen a 200 million dollar business. Approximately 864,000 dairy cows on 101,000 Michigan farms produce nearly 6 million pounds of milk annually. The average cow produces yearly about 6,470 lb. of milk containing 246 lb. of butterfat. To produce this volume she consumes 3 to 4 tons of hay or its equivalent in silage, ½ to 1 ton of grain, and 1 to 2 acres of good pasture. Over 56,000 cows are on the DHIA testing program, and an additional 10,000 are on Official Testing. It is estimated that 250,000 cows are being bred artificially.

More than 600 dairy plants process the state's milk supply, about 50% being sold as bottled milk. Michigan's position in the nation's dairy enterprise shows it to be 8th in total milk production; 2nd in sherbet manufacture; 4th in evaporated milk and nonfat dry milk solids; 5th in cottage cheese; 6th in butter and ice cream; 7th in bottled milk; and 9th in cheese manufacture.

A.D.S.A. guests will have an opportunity to observe automobile assembly techniques at the Oldsmobile plant, just 5 minutes distance from the campus. Fisher Body, the largest manufacturer of automobile bodies, is located in nearby Lansing. Detroit, the motor city; Flint, noted for automobile assembly plants; Grand Rapids, the furniture capital of the world; Kalamazoo, home of numerous paper mills; and Battle Creek, birthplace of breakfast foods, are well worth a visit.

Publicized as a "Water Wonderland," Michigan attracts millions of vacationists each year. The state is bounded by four of the Great Lakes and possesses thousands of inland lakes and a large number of state-maintained parks. For the historian, the copper country of the Upper Peninsula; the Soo locks, now celebrating their centennial year; the Straits of Mackinac; and Greenfield Village will provide ample exploration sites.

News from the University of Georgia

The Annual Dairy Production Short Course was held on Feb. 1, at Tifton, Georgia, and was repeated at Griffin on Feb. 2 and at Athens on Feb. 3. Out-of-state members of the faculty were W. E. PETERSEN of Minnesota, C. B. BENDER of New York, and MRS. SUE ANDERSON of the Chattanooga Dairy Council.

Construction of the Kellogg Continuing Education Center, which will cost over $2,000,000, began on the Georgia campus in January. The Center will open in the fall of 1956 and will include rooms for 300 overnight guests, 500-capacity dining room, 400-capacity auditorium, 27 conference rooms; radio, moving picture, and color television studios, and 750 parking spaces. The building will be air-conditioned throughout.

H. B. HENDERSON has been credited by K. L. DEVRIENDT, director of the Belgian National Milk Office, with getting the glass of milk on its way to becoming the national beverage of Belgium. In a recent article in *La Lanterne*, a leading newspaper in Brussels, Devriendt reports: "Belgium now has 68,000 dairies producing pure milk, compared with 33,000 when Henderson first arrived . . . Production per cow is also increasing . . . Mr. Henderson rendered us great services. His dynamism, his method of work with fixed objectives, and his great experience were factors which permitted us to attain, in the milk industry, very optimistic results." Henderson spent a part of 1949-50 in Belgium as technical assistant to the Belgian dairy industry for E.C.A.

Professor Henderson recently visited a number of western states in a study of dairy instruction in land grant colleges, sponsored by the Milk Industry Foundation. He also visited with industry groups in Corvallis, Ore., San Francisco, and Los Angeles.

Southern States Claim Public Health "Firsts"

The yellow fever outbreaks were the great teachers in public health in America, and many of our seaport city health departments were organized to combat the disease. Methods developed in the South have become a part of recognized public health procedure throughout the United States. Some of these innovations include:

Virginia, Jamestown—the first public health laws in the United States, enacted a few years after the founding of the colonies.

South Carolina, Charleston—the first health officer, appointed during the days of the colonies.

Maryland, Baltimore—the first city board of health.

Alabama—the first state to have all counties "covered" by public health services.

Kentucky—the first state with a full-time staff in a local health unit.

North Carolina—the first rural health unit.

Mississippi—the first county health officer.

May, 1955, marks the 100th anniversary of the Louisiana State Board of Health, oldest state board of health in the United States. To celebrate the event, the Southern Branch of the American Public Health Assoc. and the Louisiana Public Health Assoc. will meet in New Orleans at the Jung Hotel, May 10-13. Those attending will include physicians, nurses, sanitarians, engineers, health educators, nutritionists, biologists, laboratory workers, medical social workers, and voluntary health agency workers.

Technically Trained People in Industry Register Complaints

In a recent article in *Chemical and Engineering News* (March 14) A. M. ZARENS writing on "Are Engineers People?" reports: "Right or not, engineers and scientists feel they are different, and management must recognize this if it wants to get the most out of its professional people." The author reports the results of a recent survey by the National Society of Professional Engineers, which can be summarized as follows:

29% feel the company does not recognize their professional status.

30% state the company is not using them most effectively.

35% are dissatisfied on at least two of the following counts: salary, prospects, nature of work, working conditions, location of work.

45% are dissatisfied with their present salaries.

50% say they are not kept informed of their personal progress.

This is not a new discovery as far as labor is concerned. Those who have studied the causes of unrest and dissatisfaction among factory workers have long known that proper recognition must be given to human relations in industry. However, little consideration has been given to the problem as it relates to the research worker in industry. The present shortage of students at both the undergraduate and graduate level in all scientific fields should cause management to realize that there is something basically wrong with the manner in which technically trained college graduates are being treated in industry. The situation probably will get worse before it gets better, for industry's need for technical men is increasing much faster than a new supply is being created. The cupboard is practically bare as far as dairy technologists are concerned. It is a problem that deserves priority consideration.

Illinois Industry Raises Scholarship Funds

To encourage more high school graduates to seek their fortunes in the field of dairy technology, individual dairy plants throughout the state of Illinois have contributed $1,000 to $4,000 each to help finance a series of $1,000 4-year scholarships at the University of Illinois. To better acquaint high school seniors with the opportunities in the field of dairy manufactures a Dairy Technology Career Day was held at the University April 15, at which time the availability of twenty scholarships was announced. Plant operators cooperated by bringing seniors from their local high schools to the meeting. The students were taken on a tour of the campus, and at the luncheon short talks were given by President LLOYD MOREY, Dean L. B. How-

ARD of the College of Agriculture, and other university officials.

Scholarship Fund Established at Minnesota

Starting next fall several scholarships will be offered to high school seniors and college students interested in pursuing a course of study in the field of dairy industry at the Univ. of Minnesota. Various companies and individuals interested in encouraging the training of students are contributing to this fund, to be known as the Minnesota Dairy Industry Scholarship Fund. Selection of scholarship winners will be based upon academic and professional aptitude, character, and financial need. Each scholarship will amount to a minimum of $300 annually and may be renewed on application.

Klenzade Holds Seminar

A record crowd of over 700 people attended the 18th Educational Seminar held by Klenzade Products, Inc., at French Lick, Ind., March 23-26. In the opening address, A. L. SHOGREN, chairman of the board stated, "The purpose of the seminar is to promote better sanitation practices and methods." Topics of interest to those in the dairy industry included Sanitation Chemistry, Sanitation Bacteriology, Dairy Plant Sanitation, Food Poisoning, Sanitation Education, Corrosion of Food Equipment and Utensils, Dairy Farm Sanitation, and Special Cleaning Procedures. There were 119 guest speakers and 32 staff members who participated in the seminar prgram. The guest speaker at the banquet was FRANK H. BEACH, professor of marketing at the Univ. of Illinois.

AIBS Establishes New Office

As of March 15, the offices of the American Institute of Biological Sciences will be located at 2000 P St., N. W., Washington 6, D. C. The AIBS is a voluntary nonprofit organization of professional biological societies and individuals that have a common interest in the life sciences. Organized in 1948 within the framework of the National Research Council, the Institute is now assuming an independent status. Over 37,000 biologists and 30 societies are represented. Sixteen commercial concerns have joined as Associates and are contributing to the support of biological programs.

The Institute assists biological societies in national projects and problems and fosters cooperation between all societies and persons engaged in research, teaching, or the application of biology. Liaison is maintained between AIBS and the National Academy of Science–National Research Council, Federation of American Society for Experimental Biology, American Assoc. for the Advancement of Science, American Chemical Society, American Inst. of Physics, American Geological Inst., and many other scientific organizations.

The Institute arranges a convention for biological societies each year in early September on a university campus. These meetings draw 2,000-4,500 biologists, and over 1,000 research papers and symposia have been presented at each of the last three meetings. Field trips, excursions to regional research laboratories, a scientific exhibit, and special lectures and symposia are features.

The official publication of the Institute is the *AIBS Bulletin*, a quarterly magazine with a circulation of 16,000. It does not publish research results but contains articles of a general nature, news, book reviews, and reports on national and international congresses and conferences. The Institute maintains a national placement service, making available twice yearly to prospective employers a register of available biologists.

Currently the AIBS is cooperating with the National Research Foundation in the establishment of a part of the National Register of Scientific and Technical Personnel. Over 40,000 questionnaires have been mailed to biologists and the resulting information filed and coded for the future use of the government, participating societies, and responsible organizations. The AIBS is represented on the Scientific Manpower Commission and UNESCO. The American Dairy Science Association is an Affiliate Society of AIBS.

L. V. Wilson Honored by Minnesota Breeders

L. V. WILSON, Owatonna, Minn., at present consultant and advisor with the Consolidated Breeders Coop., was honored by the Minn. Livestock Breeders Assoc. at their annual meeting Feb. 10, at University Farm, St. Paul. The association, in recognition of Mr. Wilson's years of outstanding service in the field of livestock work, hung his portrait in Peters Hall at the University.

Mr. Wilson was formerly dairy specialist with the Minn. Agricultural Extension Service. He left this work to become manager of G. N. Dayton's Boulder Bridge Farm, where he developed the herd of purebred Guernseys and many national champions were produced. He served this farm enterprise from 1926 to 1950.

In recent years Mr. Wilson has been the official classifier for the American Guernsey Cattle Club. He has been a director and vice-president of this club and president of the Minn. Guernsey Cattle Club. He is now secretary of the state organization.

Fitch Recognized by Kansas Dairymen

J. B. FITCH, head, Dept. of Dairy Husbandry, Univ. of Minnesota, was honored by the State of Kansas Inter-Breed Dairy Council and the dairymen of Kansas at the State Agricultural College, Manhattan, Feb. 8. He was presented with a Certificate of Recognition, and his picture will be hung in Waters Hall, the main agricultural building at the College.

Pennsalt President to Retire; Drake Named Executive Vice-President

G. B. BEITZEL, president of the Pennsylvania Salt Mfg. Co., has expressed to the board of directors his desire to retire as chief executive later this year when he completes 25 years of service with the company. He became the 13th president of this 104-year-old company in 1949. His many business and community activities include board memberships with the Pennsylvania and Philadelphia Chambers of Commerce, Fidelity-Philadelphia Trust Co., Delaware County and Methodist Hospitals, and the Crime Prevention Assoc. of Philadelphia.

Mr. Beitzel has recommended that W. P. DRAKE be appointed executive vice-president and elected to board membership. Although only 42 years of age, Mr. Drake has been a member of the Pennsalt organization for 21 years. He was first employed as a student trainee while in college. After 3 years of technical training in several of the company's industrial chemicals plants, he was assigned to the sales organization. He served successively as sales manager of the chemical specialties department, assistant vice-president, and vice-president in charge of sales. He became president of the industrial chemicals division in 1954.

Holm Relinquishes Section Headship

G. E. HOLM, who had been head of the Dairy Products Section (formerly Dairy Products Research Laboratories) in the USDA since 1942, has been appointed special assistant to the chief of the Eastern Utilization Research Branch. In this newly-created position, Dr. Holm will round out his long career in research in the department by recording the results and extensive knowledge acquired in his experience in dairy research. He will be available also for consultation on matters pertaining to research on dairy products. E. O. WHITTIER is acting as head of the Dairy Products Section.

At the beginning of February, the Dairy Products Section, together with other sections of the former Washington Utilization Research Branch, became a part of the Department's Eastern Utilization Research Branch. No physical move was involved in this transfer, and the sections will remain at their present locations, in or about Washington. The new address is Dairy Products Section, Eastern Utilization Research Branch, Agricultural Research Service, USDA, Washington 25, D. C.

Oregon State Changes Department Name

The name of the Dept. of Dairy Husbandry at Oregon State College has been officially changed to the Dept. of Dairying. This was done to better depict the various curricula offered by the department. A new option in Milk Industry Management is being offered next fall for the first time. This will allow a student to elect one of three options, Milk Production, Milk Processing, or Milk Industry Management within the department. Students taking the new option will take the first 2 years in common with the milk processing students and then will take 40 term-hours of business courses along with selected other courses during the junior and senior years. This allows the student to take advantage of course offerings in the School of Business and Technology.

"Quality Dairy Products Sell" was the theme of the Oregon Dairy Industry Short Course and 44th Annual Convention held at Corvallis, Feb. 14-17. About 450 persons attended the 4-day meeting. Out-of-state speakers included H. B. HENDERSON, Univ. of Georgia; N. S. GOLDING, State College of Washington; W. L. DUNKLEY, Univ. of California; N. C. ANGEVINE, St. Louis, and C. B. A. (BILL) BRYANT, Chicago.

The program for the final day was devoted to advertising, merchandising, and promotion. Speakers for this portion of the program were headed by RAY ALBERTS, membership director, and F. R. NEU, publicity director, A.D.A., Chicago. R. J. WERNER, executive director, Milk Industry Foundation, Washington, D. C., was the feature speaker at the banquet held the final evening and attended by 325 persons. ARTHUR HURLBURT, Portland, was elected president of the group for the coming year, replacing GORDON COLEMAN, Eugene, the retiring president.

New Officers Elected by Southern Division of A.D.S.A.

The annual meeting of the Southern Division of A.D.S.A. was held at Louisville, Ky., Feb. 7-9 in connection with a meeting of the Assoc. of Southern Agricultural Workers. Forty papers on various phases of dairy manufacturing and dairy production were presented, and a joint session was held with the Agricultural Economics and Animal Husbandry Sections of the A.S.A.W. At the banquet on Feb. 7, F. H. HERZER, head of the Dairy Dept. at Mississippi State College, was honored for the outstanding work he has done during the many years he has served the dairy industry.

The officers elected for the current year are: W. A. KING, Clemson College, president; H. C. OLSON, Oklahoma A & M College, vice-president; and K. M. AUTREY, Alabama Polytechnic Inst., secretary-treasurer.

Oklahoma A. & M. Honors Goley

R. T. GOLEY, manager of the Gold Spot Dairy, Enid, Okla., was selected to receive the first annual award as the man who has made the most outstanding contributions to the dairy industry of Oklahoma. The recipient's picture will be hung in the dairy building at Oklahoma A. & M. College. Mr. Goley has been very active

in church, civic, state, and national organizations. Since he became manager in 1931, the Gold Spot Dairy has grown to one of the largest cooperative dairy plants in the country.

Milk Industry Foundation Teaching Award

BRUCE BALDWIN, president of the Milk Industry Foundation, has recently notified President L. A. MOORE of A.D.S.A. that the Foundation will sponsor an award of $1,000 to be given every other year to an outstanding college teacher in the field of dairy manufacturing. The winner for 1955 will be announced June 22 at the annual meeting at East Lansing.

A.D.S.A. to Become an Affiliate Society of A.A.A.S.

The American Assoc. for the Advancement of Science has announced a change in status of A.D.S.A. from Associate to an Affiliate society. On the basis of 62 of our members being Fellows of the Association we are entitled to have one A.A.A.S. council representative. President Moore will appoint this representative. A letter will be sent by the A.A.A.S. administrative secretary to all members of A.D.S.A. informing them of this change of status.

DISA Elects Officers

At the 36th annual meeting, March 3-4 in Chicago, current officers of Dairy Industries Supply Assoc. were reelected, and all incumbents in positions on the board of directors were retained with the exception of C. A. WOOD, Cherry-Burrell Corp., who has largely retired from activities with his firm. L. N. LUCAS, Bastian-Blessing Co., Chicago, was reelected president; S. E. CROFTS, Batavia Body Co., Batavia, Ill., remains as vice-president; and R. E. CAIRNS, Waukesha Foundry Co., Waukesha, Wis., was retained as treasurer. J. H. MULHOLLAND, Milford, Del., is DISA's Honorary President-for-Life.

J. G. CHERRY of Cherry-Burrell Corp. was elected a director at large, and the incumbents retained are: directors at large—T. A. BURRESS, The Heil Co., and P. K. GIRTON, Girton Mfg. Co.; commodity directors—F. M. KING, Wyandotte Chemicals Corp., for chemicals and refrigerants group; R. B. WILHELM, Owens-Illinois, for containers group; and L. N. LUCAS for point-of-sale materials group.

Announce Judges for International Dairy Show

Officials of the International Dairy Show, to be held in the International Amphitheatre, Chicago, Oct. 8-15, have announced the slate of judges who will officiate at this year's event. G. E. RAITHBY, of Guelph, Ont., will judge Ayrshires; G. W. TRIMBERGER, Ithaca, N. Y., Brown Swiss; DELBERT KINGSTON, Cary, Ill., Guernseys; W. K. HEPBURN, JR., Dalton, Pa., Holsteins; B. J. GRIFFIN, Danville, Ky., Milking Shorthorns; and HILTON BOYNTON, of Durham, N. H., and W. E. WEAVER, of Phoenix, Ariz., Jerseys.

The same judges will also pass on the junior classes of each breed, which will be judged prior to the open show. A new regulation of the Intern. Junior Show this year is that the judge will nominate entries for the open classes at the same time that he places each junior class.

At a recent meeting of the directors of the Intern. Dairy Show, W. E. WINN, president of the Pure Milk Assoc., Chicago, was elected to the board.

Department of Health, Education, and Welfare, Food and Drug Administration, Proposes Samsoe Cheese Standard

The proposal submitted by the Denmark Cheese Assoc., 17 Battery Place, New York City, is as follows:

"*Samsoe cheese; identity. (a)* Samsoe cheese is the food prepared from milk and other ingredients specified in this section, by the procedure set forth in paragraph *(b)* of this section, or by another procedure which produces a finished cheese having the same physical and chemical properties as the cheese produced when the procedure set forth in paragraph *(b)* of this section is used. The shape of the cheese is flat cylindrical. Its weight is approximately 30 lb. (14 kg.); its diameter is approximately 17 in. (44 cm.); and its height is approximately 4 in. (10 cm.). It has a small amount of eye formation of approximately uniform size of about 5/16-in. (8 ml.). It contains not more than 46% of milk fat. If the milk is not pasteurized, the cheese so made is cured at a temperature of not less than 35° F. for not less than 60 days. The surface may be covered with plain or colored paraffin or other tightly adhering coating.

"*(b)* Milk, which may be pasteurized or clarified or both, and which may be warmed, is subjected to the action of harmless lactic-acid-producing bacteria, present in such milk or added thereto. Harmless artificial coloring may be added. Sufficient rennet (with or without purified calcium chloride in a quantity not more than 0.02%, calculated as anhydrous calcium chloride, of the weight of the milk) is added to set the milk to a semisolid mass. After coagulation the mass is cut into small cube-shaped pieces with sides approximately 3/8-in. (1 cm.). The mass is stirred and heated to about 102° F., and so handled by further stirring, heating, dilution with water, and salting as to promote and regulate the separation of curd and whey. When the desired curd is obtained, it is transferred to forms permitting drainage of whey. During drainage, the curd is pressed. After drainage, the curd is removed

from the forms and is further salted by immersing in a concentrated salt solution for about 3 days. The curd is then cured at a temperature of 60° to 70° F. for 3 to 5 weeks to obtain the desired eye formation. Further curing is conducted at a lower temperature.

"*(c)* For the purposes of this section, the word "milk" means cow's milk, which may be adjusted by separating part of the fat therefrom or by adding thereto cream or skimmilk."

Virginia Holds Conferences

Fifty-four members of market milk and related industries attended the first annual Market Milk Conference and Clinic held at Virginia Polytechnic Inst. in February. The theme of the conference was a survey of the important problems encountered by the milk processor with the introduction of new equipment.

The third annual Ice Cream Conference and Clinic was held March 2-3 with 30 persons present. Lectures dealt with the manufacture, storage, and merchandising of ice cream. Clinic sessions were devoted to the evaluation of ice cream samples submitted by companies sending representatives to the meeting.

Speakers for the conference included S. J. WERBIN, Stein-Hall Co., New York City; W. M. ROBERTS, head, Dairy Mfg. Section, N. C. State College; C. W. ENGLAND, England Labs., Washington, D. C.; HARRY FISHER, Corn Products Sales Co., New York City; W. S. BRANK, Bessire Co., Richmond, Va.; J. W. POLLARD, Peoples Service Drug Stores, Washington, D. C.; G. I. FORD, The Vendo Co., Kinston, N. C.; G. C. GRAF, head, Dept. of Dairy Husbandry, V.P.I.; and E. J. FINNEGAN and G. F. NAGEOTTE of V.P.I.

Ohio Proposes Milk Control Bill

A milk control bill, which may be far-reaching in its effect, has been introduced into the Ohio legislature. It would establish a milk control commission and could control the price of milk paid to the producer as well as the price paid by the consumer. The bill was introduced in a skeleton form and will be filled in by amendment when heard before the Agriculture Committee in the Senate.

Other bills of interest to the dairy industry also have been introduced; two would transfer the inspection of Ohio's milk supply from the Dept. of Agriculture to the Dept. of Health; one would legalize milk dispensers; one would adopt the model Food and Drug Statute to the Ohio Code, another would prohibit storage of frozen foods in ice cream cabinets belonging to frozen dessert manufacturers, one lowers the fill mark on milk bottles, and another bill would legalize the 1/3-qt. container.

The Ohio Dairy Products Assoc. has been reorganized so that instead of four separate state organizations, there now is just one.

The second annual high school "Career Day" was held April 16. The Ohio Dairy Products Assoc., the Dairy Technology societies, and the Ohio Dairy Boosters Assoc. are cooperating with the University in bringing high school students to the campus to learn more about the field of dairy technology. Last year 104 students attended the meetings.

Completed Theses

M.S. Degree:

CHARLES V. MORR—Some heat-produced acids of skimmilk. The Ohio State Univ.

Ph.D. Degree:

VERNON L. BALDWIN—Mammary gland measurements in calves and their prediction value. Univ. of Minnesota.

ARTHUR L. BRUNDAGE—The utilization of smooth bromegrass *(Bromus inermis)* under rotational and strip grazing systems of pasture management. Univ. of Minnesota.

Correction in Our Industry Today Article

On page 110 of Vol. 38 (January) of the Journal in the article, "A System of Continuous Buttermaking," where the procedure for calculating total fat lost during separation is given, the author says it should have been indicated that the weight of the product in each case is multiplied by the fat test to obtain the pounds of fat lost.

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Selection and Evaluation of Dairy Products

A course offered at Michigan State College for Hotel Administration, Restaurant Management, Food Distribution, and Home Economics Majors

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Seldom do dairy instructors have such a golden opportunity to discuss and demonstrate qualities in dairy products and the factors affecting them before a segment of nonagricultural students as that offered by the course "Selection and Evaluation of Dairy Products" at Michigan State College.

This course, now in its 17th year, serves majors in hotel administration, restaurant management, food distribution, and home economics. Agricultural and dairy students are not permitted to enroll in the course. A marked increase in enrollment has been noted since World War II, and class attendance now approaches 100 students yearly.

At first, major attention was given to the four dairy products: butter, American Cheddar cheese, milk, and vanilla ice cream. Presently, by student request, attention is also given to other cheese varieties and to other dairy products. Also, the mechanics of the course have been changed to accommodate the increase in enrollment. Expediency has dictated elimination of the individual-student laboratory exercises, much as they are desired, in favor of general, pertinent class demonstration and participation. Even by scheduling two sections, the numbers enrolled were too great for the available laboratory facilities. Meanwhile, the college credits for the course have been increased from two to three term credits. The student-instructor contact time consists of two 1-hour lecture periods and one 2-hour laboratory period per week.

Geographic Areas Represented

Although students come from virtually every state in the Union and from several foreign countries, approximately half of them are from Michigan. In 1954, 54.6% were from Michigan, mostly from the larger cities; the remaining students were from 18 other states and one foreign country—Mexico. Heretofore, students have been enrolled from France, Switzerland, Italy, Greece, and Norway. Indeed, the geographical, racial, and cultural background represented on the class roll would seem to be in keeping with the wide range in variety and the universal use of dairy products.

Objectives

With the chance that others might be interested in the possibilities of a similar course, some brief pertinent information concerning the objectives and subject matter is presented here. Also, some sidelights on teaching problems and student response are given.

After class orientation, the general objectives of the course are presented and discussed at considerable length. The primary objectives are as follows:

1) *To acquaint the student with the qualities of dairy products*, so that he may *(a)* buy dairy products more economically and *(b)* be able to use the selected dairy product to the best advantage. Generally speaking, students are not aware that about one-fifth of the food dollar goes for dairy products. It seems advisable to know something about the products for which this large amount of money is spent. Also, the specificity of dairy-product use is enlightening. For example, the following questions never fail to induce curiosity:

   a) Which cheese is best suited for grating? for smörgåsbords? for toasted-cheese sandwiches? for macaroni?
   b) Why does a certain milk frequently curdle in making gravy?
   c) What does butter contribute to hot biscuits and country-fried chicken?
   d) Why is butter not best suited for deep-fat frying?

2) *To give the student an opportunity to understand the steps involved in processing dairy products* so that he may *(a)* maintain the purchased quality of the product until the product is used and *(b)* appreciate more fully the items which enter into the cost of the product.

3) *To become a fair layman-judge of dairy products.*

4) To help the hostelers, restaurateurs, and food handlers to gain confidence, and hence satisfaction, in their dairy food experiences.

5) To create a desire to use more dairy products. This objective may smack of ulterior motives. Students are so advised. However, this possible objective is incidental—a by-product—to the course.

In the summation of the objectives, other minor ones are pointed out. The class is assured that the course is given for them—not to give the instructor teaching experience nor to furnish him with the opportunity to "beat the bushes" for dairying, but that they may take away useful information.

Specific Dairy Information Desired by Students

Soon after a full discussion of the above objectives, the students are given an opportunity to express their desires for specific information on dairy products. The subject matter sought varies little from year to year. A classification of their specific requests for information on each of the dairy products last year is as follows:

a) Definitions and standards.
b) Market grades, types, and varieties.
c) Analyses; food value, vitamin content, etc.
d) Availability; season, or time to purchase; regional popularity; and best sources of supply.
e) Selection techniques; tests for quality; taste and appearance.
f) Retail and wholesale prices; costs; economy of purchase; price of competing foods.
g) Processing procedures; possibility of hotel and restaurant production and handling.
h) Sanitation techniques in processing and packaging.
i) How to display and store.
j) Selection of products for specific occasions; how to serve; possible use of cheap, but good, substitutes.
k) Production especially suited for hotel work.
l) Quantity buying.
m) Consumer demand.
n) Methods of distribution.

Considering that information on the above items is desired generally on each dairy product—butter, domestic and foreign varieties of cheese, creamline milk, homogenized milk, chocolate milk, table cream, whipping cream, half-and-half, cultured cream, yoghurt, cottage cheese, sweetened condensed milk, buttermilk, dry milk, nonfat dry milk solids, frozen desserts, evaporated milk, and ice cream—one is overwhelmed at the stupendous task of covering the subject adequately. Since the students "ask for it," the subject matter may well be presented as thoroughly as time permits, following closely the chosen subjects as outlined above.

However, before launching upon the full discussion of each product, the instructor must give certain basic dairy fundamentals, milk analysis, and terminology so that a common understanding may be had in discussing the various aspects of the numerous products.

Some Teaching Observations and Techniques

Despite the first interest and enthusiam shown by the students, they may at times become indifferent to routine lectures. Distractions at the beginning of lectures, such as good camaraderie resulting from group consciousness, the student daily paper, or an occasional crossword puzzle, may steal valuable time from the instructor if he is not alert. In such cases, McCluskey's "principle of readiness" or "conditioning" of the student for instruction must be employed. Quite effective to this end is the 5-minute quiz, though this can be overdone. One pedagogic trick that succeeds beyond all expectation is that of resorting to an irrelevant examination. When the test is properly used, its effect may well last for several days—or even weeks. Man's insatiable curiosity and ego are back of its success. The examination is carried out about as follows: Pass out ordinary filing cards requesting neither name nor seat number. Caution against group information as you wish to test the "individual-knowledge level" of the class. Ask four questions involving short answers. The following set produced no end of curiosity (and arguments):

a) Who is generally considered to be the greatest thinker America has ever produced?
b) Identify Kettering.
c) Who was president of the United States during World War I? (Several reported Franklin D. Roosevelt.)
d) Who was Schine?

The responses to such a quiz may well serve as the beginning of later lecture periods by drawing the attention of the class to a sharp focus for a continuation of dairy-product instruction. The size of the class virtually dictates use of the lecture method of presentation during the 50-minute period. Nevertheless, a limited number of bona fide questions must be entertained when they arise.

One must bear in mind that teaching a class of nondairy students is an opportunity to serve them—not "to cover the waterfront." If teaching has been done well, then some of "the rain on the pulpit will drip into the pew" and dairying will have been served. To this end, the instructor must be aware also of McCluskey's "principle of Gestalt," a German word meaning that "the whole is more than the sum of the parts." Thus, it is important for the instructor of a nondairy class always to keep in mind the larger picture while describing the parts and details.

The course will be effective if the teacher points out the connection between one situation and another or illustrates it in terms of the student's experience. For example, the mere description and definition for consumer grades and government scores of butter, although of interest, lack classroom punch in themselves. But when a Grade AA, 93-score butter is correlated with a $5.00 steak dinner, then the quality of butter takes on a new meaning, which they readily comprehend. Especially are they receptive when it is pointed out that good butter makes dinner-patron satisfaction and literally "freshens and tenders" the $5.00 steak in the eater's mind—and all this at a product cost of about 1¢ per pat. Then the inevitable question: Why not serve two pats? three? or a sixteenth of a pound? The cost is negligible. Be assured the class is 100% receptive when it is explained further that Grade AA, 93-score butter (give them a pat to eat meanwhile) costs only about 1/20 of a cent more per pat than a similar quantity of 89-score butter—and makes the $5.00 meal more complete. Admonish them: "Know your butter! Select the finest. What kind of restaurant will you have? Just another 'joint' or a fine eating establishment?" Silence—the student answers only to himself. The "principle of Gestalt" has been effective.

Laboratory Exercises

The laboratory exercises for a course in the selection and evaluation of dairy products actually make the course. Here the instructor may demonstrate, illustrate, and amplify his lecture material. Possibilities for class exercises are unlimited. The following effectively used exercises suggest many others:

1) Identifying substances by olfactory sense.
2) Movies (M.S.C.'s "Sentinels of Milk," ADA's "Miracles of Milk," Kraft's "Cheese Family Album," etc.).
3) Techniques in grading.
4) Discussion of preparation of term paper or speech outline.
5) Study of dairy products served on outstanding hotel and restaurant menus.
6) Quality demonstrations for each dairy product.
7) Standardization of milk.
8) Classification of cheese.
9) Judging classes of several dairy products.
10) Cheese or dairy festival.

From the above list, one surmises that the instructor need not resort entirely to "spoon feeding" the students but can require them to make some contribution. Several possibilities are evident, but the most satisfactory contributions are those in which the student literally "loses himself" for the cause. Requiring a term paper is a student "joy-killer" and often a waste of time. Frequently, the assignment is done under compulsion, and the possibilities of fraternal collusion are not overlooked. However, the same goal (getting a student to dig out dairy information to serve his needs) may be accomplished by requiring the student to draw up a complete speech outline on some phase of dairying of value to him. Obviously, in the time allotted for the course, a hundred 10-minute prepared speeches cannot be given. Nevertheless, each student may be impressed with the fact that his speech might be the chosen one. Furthermore, he can be encouraged to make the outline of the dairy speech so good that it can be used in his speech course. This possibility appeals to the students. And, in addition, the instructor knows that in this manner good dairy information is being disseminated in ever-widening circles.

The Cheese Festival

Perhaps the most effective student-contributing laboratory exercise in connection with this course is that of staging a "Cheese Festival" at the close of the term. This becomes a gala event in which students, staff, and guests meet to partake of the festivities and to sample the numerous varieties of domestic and foreign natural cheese. For the most part these cheeses are sought by the students for their rarity and quality and brought by them in the hope either of winning a prize or of "stumping" the judge who must classify them.

FIG. 1. Student attention is spontaneous at the Cheese Festival.

The Cheese Festival is the climactic point of the course—made so largely by spirited student participation. Students are not required to attend the Festival (none has absented himself yet), but if they do attend they are required to supply, two days prior to the event, a substantial portion of a natural cheese of any variety for judging and classifying. They may remove or change the wrappers and any identifying marks so as to check on the judge's classifying ability. Previously, the students have been challenged and defied to submit a cheese that the instructor could not classify. Experience has shown that it is good pedagogy to admit defeat—to let the student "put one over on the prof." (Frequently, the "prof" cannot do otherwise.) Thus, the winning student becomes

a hero in the eyes of his classmates, and the cheese itself, a rare specimen, results in an unscheduled discussion of its qualities. Likewise, the highest quality cheese in each class is pointed out and the winner announced. Finally, the sweepstakes award is made. Then the class and guests are free to examine the cheese and eat at will.

FIG. 2. Display of cheese at the 1954 Cheese Festival.

Scheduling a cheese festival requires much time and effort, both in planning and in staging. However, the students are a real help. Heretofore, they have volunteered to decorate the room, make the coffee, purchase the rye bread, arrange samples, cut the butter, invite guests, and do dozens of chores incident to the occasion. Campus and off-campus guests lend dignity to the affair. They are indeed guests—extended full courtesy, introduced, served first, allowed to inspect the cheese show and listen to the class discussion.

Such an exercise could not be carried out without industry, college, and student cooperation. However, the results suggest the effort is worthwhile. Two unsolicited general reactions to the Cheese Festival seem significant: *(a)* students learn cheese classification in minute detail (that is the chief objective of the festival), and *(b)* some of the guests from the extension division catch the vision of the possibility of using this technique to acquaint consumers with the various qualities of several kinds of cheese (this has been done on several occasions).

Some Consumer Reactions Noted

Learning in this course is not in one direction only. The instructor gets a cross-section pattern of consumer thinking on various products and phases of the dairy industry not generally gleaned elsewhere. For instance, early sympathies of the class seem to be definitely for margarine. Seemingly, nothing would please the class more than a harangue on the butter vs. margarine controversy. So far as the instructor is concerned, margarine is not even in the teaching picture of this course. If depreciated at all, it is only through a neutral, apathetic concern for the product. Likewise, the instructor has nothing against lard, but lard-making and its uses are taught in the Animal Husbandry Department. Good butter is talked up, demonstrated, and its contribution to good eating pointed out. No one gains when another product is decried.

FIG. 3. Food distribution, restaurant management, hotel administration, and home economics majors examine cheese.

Another sensed class belief is that the price of milk is too high; the delivery system is costly. There is no use to argue against set minds and opinions. But a simple, before-their-own-eyes demonstration of filling an empty milk bottle with five bottles of a popular "inexpensive" beverage and letting them do a little mental arithmetic is surely convincing. Nothing further need be said about the high price of milk.

A term paper revealed another interesting sidelight not heretofore considered by the dairy industry. The student had interviewed a hotel manager as to what, when, and how often dairy products were included on the menu. Puzzling was the fact that cottage cheese was served but once a week. Why? "The price of pineapple was too high."

General Observations

A comment or two on the general nature of the class personnel over the years would seem to be in order. Two striking observations are made: *(a)* group consciousness prevails with certain segments of the class and *(b)* the class knows little about the dairy industry—except possibly misinformation and a negative attitude toward it. These conditions together furnish a challenge to a dairy instructor which is not often experienced in teaching dairying to dairy or to sympathetic agriculture students. In fact, these behavior patterns by apt students in a course outside their chosen field almost lead an instructor to believe that the students defy him to teach them. Nevertheless, when the students are "broken to lead," they come to the educational "trough" with an eagerness and

enthusiasm which would delight the eye even of a Mr. Chips. This manifestation toward interest in the subject and thirst for dairy facts must be turned to good account and must never be entirely quenched—even at the end of the term.

Completely different techniques than those commonly employed in teaching dairying to dairy students must be employed. The instructor must let the students know that he is there to serve their interests—and only theirs. Utter frankness is essential. Nothing should be hid or evaded. The instructor merely is a tool by which facts supported by data are unearthed for their edification. The instructor must earn and retain the students' confidence.

Conclusion

Here is a great opportunity to teach the potential hotel and restaurant managers, and other food buyers of tomorrow the truth about dairy processing, quality selections, and the enjoyment of good food. Dairy departments having an adequate staff but limited students might well consider the potentialities of a course in the selection and evaluation of dairy products for consumer or special-purchasing groups.

Some Aspects of the Detection of Watering in Milk[1]

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During World War II, a number of Iowa milk plants requested simple procedures for detection of water added to milk, because of the fact that the high price of milk and the purchase of milk on a fat-plus-skimmilk basis again had made addition of water profitable.

Two possible methods of determining added water could be employed, the freezing point lowering or cryoscopic method (CM) and the lactometric procedure (LP). Each of these presented some difficulty, as the following discussion will indicate.

CM is considered to have an accuracy that permits detection of 5% added water. The principle of the method is the same as that applied with anti-freeze solutions, viz., solution of ethylene glycol (an anti-freeze) in water causes the water to freeze at a temperature lower than the freezing point of water (0° C.). Similarly, the dissolved and suspended materials in milk lower its freezing point, and when water is added the solids content decreases and the freezing point rises and approaches that of pure water.

There were three factors relative to CM that raised questions as to its use: *(a)* cryoscopes then were not available, *(b)* two average freezing points had been suggested for milk (−0.550° C. by the AOAC and −0.544° C. by Elsdon and Stubbs), and *(c)* two equations had been suggested for calculating added water.

I (AOAC)

$$\text{per cent added water} = [(T - T_1)/T] \cdot 100$$

II (Elsdon and Stubbs)

$$\text{Per cent added water} = [(T - T_1)/T]\ (100 - \%\ TS)$$

in which T is the average freezing point depression of milk, T_1 that of the sample, and per cent TS is the percentage total solids in the sample investigated.

Elsdon and Stubbs contend that the AOAC equation is based on the assumption that, on watering, the total water content of milk is not changed, whereas the term, $100 - \%\ TS$, in their own formula takes into account the change in water content. It remained, therefore, to determine what combination of average freezing point and equation would most nearly accurately determine the quantity of water added to milk.

As regards LP, a limiting formula had been advanced for estimating the amount of water added to milk:

III

$$\text{per cent added water} = [(SNF_1 - SNF_2)/SNF_1] \cdot 100$$

in which SNF_1 is the SNF (solids-not-fat) of the milk prior to watering and SNF_2 the SNF after watering. It has been customary to employ the legal limit for SNF (in Iowa 8.5%) as SNF_1, which, as can be seen, yields an answer with little validity.

In addition, several formulas have been suggested for determining the total solids of milk from the lactometer reading:

IV (Babcock)

$$TS = 1.1842\,F + 0.2631\,L$$

V (Babcock)

$$TS = 1.2\,F + 0.25\,L$$

VI (Richmond)

$$TS = 1.2\,F + 0.25\,L + 0.14$$

VII (Sharp and Hart)

$$TS = 1.2537\,F + 0.268\,L/S$$

VIII (Herrington)

$$TS = 1.2537\,F + 268\,(L + 3.0)/(L + 1000)$$

[1] Journal Paper No. J-2669 of the Iowa Agricultural Experiment Station. Project No. 1126.

[2] Current address: The Agricultural College of Norway, Dept. of Dairy Industry, Vollebekk, Norway.

IX (Rueda)

$$TS = 1.2537\,F + 0.268\,(L + 3.2)/S$$

X (Livak)

$$TS = 1.2537\,F + 0.268\,(L + 3.5)/S$$

In these equations F is the fat test of the milk, L the lactometer reading and S the specific gravity of the milk (in VII at 30° C/30° C., in IX and X at 30° C/15.5° C).

Milk fat progressively solidifies with time after milking, and Toyonaga indicated that this caused the specific gravity, as determined by the lactometer, to become greater as the time after milking increased. The effect of fat solidification is disregarded in Equations IV, V, and VI, which are employed with milk tempered to 15.5° C. Equations VII to X, inclusive, are employed with milk heated to 45° C., held 2 minutes and cooled to 30° C.; this treatment permits making the lactometer reading when the fat is in a reproducible (liquid) state.

The equations (IV through X) were designed for use with nonwatered milks. There was no assurance, therefore, that they were satisfactory with watered milks.

It was considered that if LP could be employed to detect watering of milk, the following questions would have to be answered: *(a)* which, if any, of the lactometric procedures and formulas could be employed to determine SNF_2, *(b)* would it be possible to evaluate SNF_1 with sufficient accuracy, *(c)* could Equation III be changed in form so that the percentage added water could be calculated with sufficient accuracy from F and L, and *(d)* assuming that 2 and 3 could not be answered with complete satisfaction, could a diagnostic procedure be evolved for calculating added water, with sufficient accuracy, by LP?

Experimental Methods

A total of 16 lots of milk were obtained between October and March. Of these samples there were two each from Holstein, Jersey, Brown Swiss, Guernsey, and Ayrshire cattle and six samples from mixed herd milk delivered to the College Dairy. A member of the Dairy Industry staff was present at each milking to be certain that no water was added to the milk accidentally or otherwise.

Total solids were determined, by the Mojonnier method, on the original milk samples and on the three portions of this milk to which water was added. Babcock fat tests (AOAC method) were determined for each of the four samples on the day the samples were prepared. Lactometer readings, total solids, and freezing points of the milk were determined on the following day.

Milk samples were stored overnight at about 40° F. They were removed from the refrigerator and treated as follows for the lactometer readings:

(a) Mixed thoroughly, adjusted to 15.5° C., and the lactometer readings obtained with a lactometer tempered to 15.5° C.

(b) Mixed thoroughly, warmed to 45° C., held 2 minutes, mixed thoroughly, adjusted to 15.5° C. (30-40 minutes), and the lactometer readings obtained with a lactometer tempered to 15.5° C.

(c) Identically as *b* except that the samples and lactometer were adjusted to 30° C. (to keep the fat liquid) and were read at that temperature.

The lactometers (Quevenne) employed at 15.5° C. (Kimble Glass Co.) were graduated from 20° to 37° Q., in 1° intervals at 15.5° C/15.5° C. Those employed at 30° C. (Rascher and Betzold) were calibrated from 13° to 28° Q., in 1° intervals at 15.5° C/15.5° C. For future fabrication, the lactometers employed at 30° C. should be calibrated from 17° to 32° Q., for greater utility. Kimble No. 20065 lactometer cylinders, 480-ml. capacity, total height 300 mm., i.d. 46 mm., were employed for all lactometer readings.

Equations IV through X were employed to calculate the percentages of total solids from F and L. In order to obtain the specific gravities at 30° C/30° C for Equation VII, the lactometers were calibrated at 30° C. in sucrose solutions. The lactometer readings then were

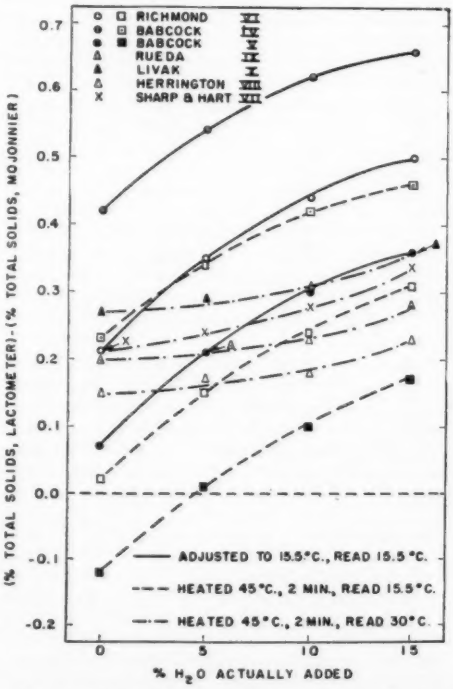

FIG. 1. Algebraic means of errors in estimating the percentages of total solids in normal and watered milk by lactometric procedures.

plotted against the specific gravities of the sucrose solutions at 30° C/30° C. The lactometers were calibrated at three points, the data were plotted, and it was assumed that the change in specific gravity scale was linear between each two calibration points.

In determining the freezing points a Hortvet cryoscope was employed. The percentages of added water were calculated by the A.O.A.C. (I) and by the Elsdon and Stubbs (II) formulas. The calculations were made with two average freezing points for milk, –0.550° C. (A.O.A.C.), and –0.544° C. (Elsdon and Stubbs). As a matter of interest, calculations were also made with the limited freezing point (0.530° C.) suggested by Elsdon and Stubbs.

Results

The Lactometric Procedure. If Equation III is to be employed satisfactorily, one must be able to evaluate SNF_1 and SNF_2 with reasonable accuracy. That this might not be done for SNF_2 was indicated by Desai and Patel, who stated that Richmond's equation (VI) yielded results that were high for normal milk and that the discrepancy increased with the amount of water added. The data of Figure 1 present the results of a study of Equations IV through VI, with methods *a* and *b* and Equations VII through X, with method *c*.

The results for *TS* with Equations IV through VI were wholly unsatisfactory. Those for Equations VII through X were higher than they should have been but did not vary greatly with increased addition of water. Since Equation VIII yielded the least variation, it was corrected as follows:

XI (modified Herrington)

$$TS = 1.2537\,F + [268\,(L + 3.0)/(L + 1000)] - 0.15$$

From this equation, SNF_2 can be satisfactorily calculated:

XII

$$SNF_2 = 1.2537\,F + [268\,(L + 3.0)/(L + 1000)] - 0.15 - F$$

SNF_1 was calculated from an equation derived from Jacobson's data for New England bulked milks:

XIII

$$SNF_1 = 0.4\,F + 7.07$$

The values of SNF_1 (Equation XIII) and SNF_2 (Equation XII), when employed in Equation III, yielded calculated values for added water that were 0.2% high for nonwatered samples and too low for the watered samples (Figure 2). This figure indicates that the error is a linear function of the amount of water

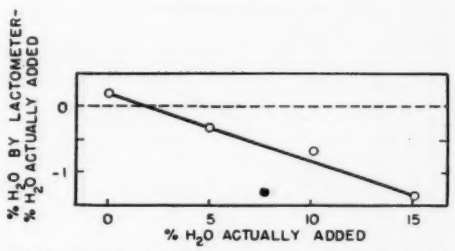

FIG. 2. Algebraic means of errors encountered when the percentage water added to milk was calculated by the formula $(SNF_1\text{-}SNF_2)/SNF_1\,100$; SNF_1 was estimated from the Babcock test and Equation XIII and SNF_2 was estimated from Equation XI.

added. It was possible, therefore, to modify Equation III as follows:

XIV

$$\%\text{ added water} = [(SNF_1 - SNF_2)/SNF_1] \cdot 100 - 0.2 + 0.1 \times \%\text{ added water},$$

which can be simplified to yield

XV

$$\%\text{ added water} = 110.89 - 111.11\,SNF_2/SNF_1$$

On the basis of the data obtained with Equation XV and known samples that were skimmed or watered, the following diagnostic procedure, based on the lactometer reading and the fat test, was evolved:

If the percentage added water calculated was:

(a) Less than –4.0%, the samples had been skimmed,
(b) Between –4.0% and –1.5%, the sample was suspected of having been skimmed,
(c) Between –1.5% and 2.0%, the sample was normal,
(d) Between 2.0% and 5.0%, the sample was suspected as watered, and
(e) Greater than 5.0%, the sample was considered watered.

The Freezing Point Method. The results obtained in this portion of the study are presented in Figure 3. The results show that the error in the result calculated by the AOAC method (Equation I) increases considerably as the percentage water added increases, regardless of the freezing point lowering used; when the freezing point lowering, 0.550° C., is employed, the percentage added water calculated is high.

The Elsdon and Stubbs formula (II) very slightly underestimates the percentage water added (0.05 to 0.30%) when the freezing point lowering, 0.550° C., is employed; the amount by which the result is in error varies but little

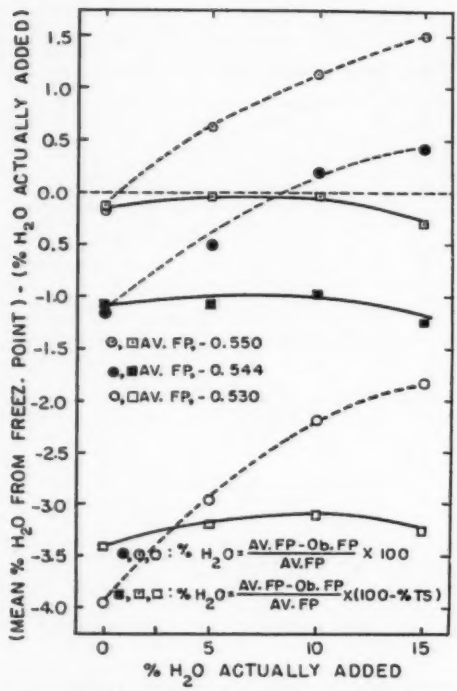

FIG. 3. Algebraic means of errors encountered when the percentage water added to milk was calculated by the AOAC and Elsdon and Stubbs equations and three freezing points for milk.

in the range 0-15% added water. The calculated result varies from the actual water added by about 1% when the freezing point lowering, 0.544° C., is employed.

These results indicate that the Elsdon and Stubbs equation (II), when employed with the freezing point lowering, 0.550° C., estimates the water added with satisfactory degree of accuracy.

Conclusions

The results that have been presented indicate that:

1) The modified Herrington formula

XI

$$TS = 1.2537\,F + [268\,(L + 3.0)/(L + 1000)] - 0.15$$

satisfactorily determined total solids in normal and watered milks produced in the Ames milkshed, when these milks were heated to 45° C., held 2 minutes, adjusted to 30° C. (30-40 minutes) and read at that temperature with a lactometer at 30° C.

It is suggested that the method be studied in other milksheds.

2) The degree of watering (and in extreme cases, skimming) can be determined by the following formula:

XV

$$\text{Per cent added water} = 110.89 - 111.11\,SNF_2/SNF_1$$

when SNF_1 is calculated as follows:

XIII

$$SNF_1 = 0.4\,F + 7.07$$

and SNF_2 is obtained by subtracting the fat percentage from the total solids calculated by Equation XI.

3) When the added water calculated by Formula XV lies between –1.5% and 2.0%, the sample is considered normal, between 2.0% and 5.0% the sample is suspected as watered, and when greater than 5% the sample is considered definitely watered.

Because of the consistency of the results obtained by the lactometer procedure and diagnostic method, the combination seems worthy of controlled study in other milksheds.

4) The Elsdon and Stubbs formula,

II

$$\text{Per cent added water} = [(T - T_1)/T]\,(100 - TS)$$

together with the freezing point lowering, 0.550° C., yielded the most nearly correct evaluation of added water among the combinations studied. The data so obtained were shown statistically to permit evaluation of added water within ±3%, 99 times in 100, when applied to the data from which these variations were calculated.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

234. Successful Dairying. C. B. KNODT. McGraw-Hill. New York. 381 pp. More than 179 illustrations—8 colored plates. 1954.

The author of this newcomer in the rural activities series for vocational students aims to provide complete information for the development and operation of a dairy herd and farm. Each of the 12 chapters presents a successive division of considerations and tasks to be done in the successful management of a dairy herd, i.e., getting started in dairying, selecting dairy cattle, raising dairy calves, dairy heifers and the herd sire, providing feed and housing, managing the dairy herd and health. The concluding 3 chapters deal with producing high quality milk and cream, marketing dairy products and cattle and finally handling dairy products in the farm household.

The outline to be followed is given early in each chapter with the chapter headings and sub-headings clearly set out in large or bold face type. All the chapters contain a list of supplemental activities at their conclusion. Cross reference on occasion guide the reader to related subject matter in other parts of the book.

Appendices include the list of agricultural colleges and their locations, USDA bulletins and leaflets on dairy farming, farm magazines, supplementary texts, a complete dairy record system and a list of visual aids dealing with dairying. Three hundred ninety-three major items are indexed by page.

A wide variety of subject material has been drawn upon to bring would-be dairy farmers the viewpoint of the author. All relevant material cannot be presented. In an act as subjective as teaching the reviewer is cognizant of the fact that a variety of subject matter orders and emphases can be expected. The reader is most likely to be impressed by the large number of high-quality photographs illustrating the many phases of the dairy farming business. The use of over 50 pages of illustrations on construction of buildings, however, will be considered too extensive by most readers. The 8 full-page colored plates are particularly striking and will serve to motivate most readers. The errors are infrequent. L. O. Gilmore

CHEESE

235. Process for making cheese from milk of low fat content. G. J. STREZYNSKI (assignor to Faster Food Products Co.). U. S. Patent 2,701,204. 36 claims. Feb. 1, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 1: 123. 1955.

Skimmilk cultured to pH 4.8 and having an acidity of about .68% is separated into curd and whey by passage through a continuous centrifugal. R. Whitaker

236. Production of heat-treated cheese products from milk of low fat content. S. SILBERMAN (assignor to Faster Food Products Co.). U. S. Patent 2,701,202. 12 claims. Feb. 1, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 1: 122. 1955.

A low fat base curd is prepared from coagulated skimmilk, by means of centrifugal force. The curd contains less than 8% fat and is further processed by heating the curd from the centrifuge to at least 130° F. R. Whitaker

237. Container. F. N. SULLIVAN (assignor to Kraft Foods Co.). U. S. Patent 2,701,091. 2 claims. Feb. 1, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 1: 94. 1955.

A rectangular-shaped box folded from a cut cardboard blank and suitable for packaging products, such as cheese, where the added protection of double thickness of board on the four side walls is preferred. R. Whitaker

238. Apparatus for trimming cheese. F. ABERLIN (assignor to Kraft Foods Co.). U. S. Patent 2,700,821. 1 claim. Feb. 1, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 1: 25. 1955.

A device is described for removing the surface layers from cheese. R. Whitaker

239. Forsoeg vedroerende udbytte—og overgangstal ved ostning af maelk fra koer af Roed Dansk Malkerace og Jerseyrace. (Experiments concerning yield and "recovery numbers" of milk from Red Danish and Jersey cows when made into cheese). H. E. BIRKKJAER, AA. H. PEDERSEN, J. L. HERMANSEN, and S. GOTTFREDSEN, State Expt. Creamery Sta., Hillerød, Denmark. Bull. 86. 1954. (English summary).

This subject has previously been abstracted from *Maelkeritidende*, **67**: 727. 1954. The abstract appeared in *J. D. S.*, **37**: A144. 1954. However, Bull. 86 is a more detailed report on the subject than the previous article and in addition to the English summary explanatory text in English accompanies each table.

T. Kristoffersen

DAIRY BACTERIOLOGY

240. The role of psychrophilic bacteria in the keeping quality of commercially pasteurized and homogenized milk. J. C. Boyd, C. K. Smith, and G. M. Trout, Mich. Agr. Expt. Station, East Lansing. J. Milk and Food Technol., **18**, 2: 32. 1955.

The keeping quality of pasteurized and homogenized milk during summer, autumn, and winter months was determined at 40 and 33° F. Psychrophilic bacteria counts and organoleptic tests were made on each sample stored at the above temperatures from 11 to 14 days.

The keeping quality of the milk was extended for 11 to 14 days by lowering the temperature from 40° to 33° F. Milk stored at 40° F. showed a psychrophilic bacteria count up to 50,000 per ml. but the flavor score was lower.

Milk collected during the autumn and winter months was poorer in quality than the summer milk samples. The difference in quality was more pronounced when the milk was stored at 33° F. than at 40° F.

There was no evidence of oxidized flavor in any of the milk samples. The deterioration of flavor was closely associated with the growth of psychrophilic bacteria in all samples.

H. H. Weiser

DAIRY CHEMISTRY

241. Separation of alpha-, beta-, and gamma-caseins from whole casein. N. J. Hipp, M. L. Groves, and T. L. McMeekin (assignors to the United States of America). U. S. Patent 2,702,800. 9 claims. Feb. 22, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 4: 564. 1955.

Whole casein is completely dissolved in an aqueous urea solution. The solution then is diluted with water to about 4.6 M urea and the precipitated α-casein is removed. Further dilution to a urea concentration of about 1.7 M causes the β-casein to precipitate. γ-casein is recovered from the filtrate by precipitation.

R. Whitaker

242. Measurement of oxidation in dried milk products with thiobarbituric acid. C. G. Sidwell, H. Salwin, and J. A. Mitchell, Jr., QMF&C Inst., Chicago, Ill. J. Am. Oil Chemists Soc., **32**, 1: 13. 1955.

A new procedure for the TBA test (thiobarbituric acid) is discussed. The advantages claimed for this revised test are (1) Freedom from physical interferences of proteins, as well as turbidity in the test solution, which is accomplished by steam distillation of an acidified slurry of milk powder; (2) Less complicated apparatus; (3) No organic solvent is necessary for the separation of the developed color from the protein. Flavor evaluations indicated a relationship existed between the flavor scores at values obtained by the modified TBA test. Reagents, apparatus, and details of the procedure are provided.

R. W. Hunt

DAIRY ENGINEERING

243. Air-cooled condensers for large tonnages. O. J. Nussbaum, Kramer-Trenton Co., Trenton, N. J. Ind. Refrig., **128**, 3: 15. 1955.

The author presents the possibilities of the remote air-cooled condenser. The advantages are listed as follows: (1) The condenser requires no water; therefore, the danger of sealing, corrosion, and winter freeze-ups does not exist; (2) There is no necessity for plumbing; (3) Maintenance cost is low; (4) The important problems of water restrictions and sewage limitations are solved as they are not used by the condenser; (5) The control of head pressure during the winter has been fully and inexpensively provided for by a simple control; (6) Both first cost and operating cost of an air-cooled condenser compare favorably with other types of condensers. The air-cooled condenser as developed for larger tonnage units is equipped with a motor-driven fan and is so provided with control that air supply can be modulated to meet the various conditions of seasonal temperature fluctuations. The air-cooled condenser operated on high air delivery is slightly less in operating cost per 1,000 ton hours than an evaporative condenser and less than half that of a water-cooled condenser.

L. M. Dorsey

244. Radiation sterilization. Review of status and problems of radiation preservation of foods and pharmaceuticals. S. A. Goldblith and B. E. Proctor, Dept. of Food Technol., Mass. Inst. of Technol., Cambridge.

This paper discusses the fundamental aspects of the effects of ionizing radiations on foods and their components and the importance of these basic effects on vitamins, amino acids, enzymes, organic compounds of biological importance, and microorganisms. Sources of energy are evaluated and an economic analysis of the process is given.

S. Patton

245. Centrifugal valve for cream separators. W. H. Harstick (assignor to International Harvester Co.). U. S. Patent 2,700,392. 1 claim. Jan. 25, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 4: 528. 1955.

Openings in the peripheral wall of a cream separator bowl are sealed by a soft rubber-like material molded to a centrifugally-operated spring valve.

R. Whitaker

246. Reclosable container. R. K. Britton (assignor of ½ to Ex-Cell-O Corp. and ½ to Ace Carton Corp.). U. S. Patent 2,702,152. 10 claims. Feb. 15, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 3: 387. 1955.

A top for a paper milk bottle, consisting of a flat top with a protected pouring lip. The pouring lip is exposed only when a flap is torn along a perforated line and folded open. The flap may be folded back as a temporary means of closing the container. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

247. Four small plants become one to challenge the top operators. W. Troy, McGraw-Hill News Bureau, Cleveland. Food Eng., **27**, 2: 63. 1955.

To better meet competition, 4 independent dairies in Columbus, Ohio merged into Harmony Farms Dairies, Inc. The organization operates as a purchasing and processing company and members retain their corporate identity so far as sales are concerned. At first, processing was done in each of the member plants, but later another location was obtained for combined processing. After working out the various organization and operating problems involved, definite benefits resulted. Reduction in the number of processing units increased efficiency 10-15%. Retail delivery truck miles have been cut 15-20%. Better utilization of labor has resulted and there has been approximately 30% savings in power. Other advantages also have resulted and gross sales have increased 5%. T. J. Claydon

248. Barriers to the interstate movement of milk and dairy products in the eleven Western states. J. S. Hillman, J. D. Rowell, and V. L. Israelsen, Ariz. Expt. Sta., Tucson. Bull. 225. 1954.

A discussion of federal, state, and local milk regulations, as they affect the free movement of milk between the eleven Western states, is contained in this publication. R. W. Hunt

249. Seasonal variations in prices, sales, and supplies of milk and feeds. P. L. Kelly, J. H. McCoy, and H. Tucker, Kan. Agr. Expt. Sta., Manhattan. Cir. 309. 1954.

An index designated as the "index of irregularity" has been established. This index is a measure of conformance of individual years to the seasonal average and is computed by averaging the absolute deviation of individual monthly percentages from the index for the month. Graphs are drawn for production, marketing, and prices of dairy products and feeds. This work was undertaken with the hope of assisting Grade A milk producers. One of the major dairy industry problems in Kansas is supplying milk to Grade A markets at a uniform rate. R. W. Hunt

FEEDS AND FEEDING

250. Studies of the secretion of milk of low fat content by cows on diets low in hay and high in concentrate. IV. The effects of variations in the intake of digestible nutrients. C. C. Balch, D. A. Balch, S. Bartlett, V. W. Johnson, S. J. Rowland, and J. Turner. Natl. Inst. Research Dairying, Univ. of Reading. J. Dairy Research, **21**, 3: 305. 1954.

Digestibility trials were conducted with 5 cows which were fed during successive periods as follows: (1) normal diet of hay and concentrate, (2) 4 lb. hay and concentrates, which did not depress fat % in milk, (3) 4 lb. hay and concentrates which depressed fat %, (4) normal hay-concentrate diet. The fat content of the milk fell from an average of 4.04 to 3.00% going from period 2 to 3 and this was reversed by going on to period 4. Solids-not-fat were definitely elevated in the period of low fat content. The major portion of the increase in solids-not-fat was due to the protein fraction. The concentrate used in Trial 2 was made up of a variety of constituents containing 16.0% crude protein, 2.9 ether extract, 50.6 N.F.E. and 9.6% crude fiber while the concentrate in Trial 3 was made up of 50% flaked maize, 35% weating and 15% decorticated ground-nut cake which contained 15.9% crude protein, 2.6% ether extract, 57.4% N.F.E., and 4.6% crude fiber. The concentrate in Trial 3 contained appreciably more digestible starch than did the concentrate in Trial 2. The authors assert that diets which depress milk fat content possess two essential characteristics. They provide a large intake of starch, or possibly other highly digestible carbohydrate, at a time when the diet lacks the physical property of fibrousness.

J. D. Donker

251. L'Instabilité du lait per suite d'une augmentation de la teneur en ions de calcium (The instability of milk because of an increase in calcium ion content). L. Seekles and W. Th. G. M. Smeets, Le Lait, **34**, 339. 1954.

The instability of milk protein known as the "Utrecht Anomalie" has been studied over a period of years. The phenomenon occurs at times of lush feed or sparse feed, as was the case during World War II, and disappears when feed conditions are nearly normal. The lack of protein stability is attributed to an elevated concentration of calcium ions in milk ultrafiltrate. Correction can be made by feeding cows 50 g. or more, of sodium citrate per day. Calcium ion concentration was determined by the Smeets modification of the Schwarzenback method. A. W. Rudnick, Jr.

252. Making compositions containing viable rumen organisms. G. A. Jeffreys. U. S. Patent 2,700,611. 5 claims. Jan. 25, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 4: 582. 1955.

A feed for livestock containing viable rumen organisms. A suspension of the organisms is

mixed with sugar and absorbed on a solid carrier such as finely ground cellulose, milk solids, etc., to form a granular mixture. To preserve the viability of the rumen organisms, the mixing is conducted in the absence of air, the granules are dried under vacuum, released to atmospheric pressure with an inert gas, and coated with a film or layer of material impervious to the atmosphere. R. Whitaker

253. Value of pastures for dairying in East Texas. S. E. CARPENTER, P. R. JOHNSON, and R. E. LEIGHTON, Texas Agr. Expt. Sta., College Station. Prog. Rpt. 1703. 1953.

As a result of treating the pastures used in this experiment, a return of $40 was realized for each $18 spent. R. W. Hunt

254. Digestibility of a sample of pasture grass by calves. D. G. ARMSTRONG, T. R. PRESTON, and R. H. ARMSTRONG. Nature, **174**, 4443: 1182. 1954.

Grass at the pre-flowering stage was cut, well mixed, and frozen in 3-4 lb. lots. Four unrelated Ayrshire male calves, age 10-12 wk. were fed the grass for 20 days in metabolism chambers. Observations were made on the calves during the last 10 days. A 2-g. capsule of Cr_2O_3 was fed each calf once a day. The mean digestibility coefficients for the 4 calves were found to be: dry matter 75.0%, organic matter 77.1%, crude protein 59.8%, ether extracts 45.6%, crude fiber 79.7%, NFE 82.9%, cellulose 84.0%, and lignin 3.0%. Some inconsistencies between calves were noted. Mean recovery of Cr_2O_3 was 102.3% ± 1.93%, showing the usefulness of this substance as a reference material in calf digestibility studies. R. Whitaker

255. The use of seaweed meals in concentrate mixtures for dairy cows. A. W. A. BURT, S. BARTLETT, and S. J. ROWLAND, Natl. Inst. Research Dairying, Univ. of Reading. J. Dairy Research, **21**, 3: 299. 1954.

Three concentrate mixtures, two of which contained seaweeds *Ascophylum nodosum* or *Laminaria cloustoni*, were fed to 18 Ayrshire cows in a complementary 3×3 Latin square design, replicated 3 times (21 day periods). The concentrate was fed at the rate of 3½ lb/gal of milk above 1½ gal. The requirements to the first 1½ gal. were met by 30 lb. kale and an average of 18 lb. hay. The seaweed meals made up 10% of two of the meals. Milk production which was in excess of 45 lb/cow/day was not affected by the seaweed meals as compared to the basal concentrate mixture. Fat percentage showed no differences between treatments. Solids-not-fat varied from group to group but showed significant variation between treatments, being highest for the basal ration. The seaweeds were appraised as having a value equal to 7 parts oat feed and 1 part common salt and were of low palatability. J. D. Donker

HERD MANAGEMENT

256. Starting farming today. Can it be done? What does it take? G. A. POND, Minn. Agr. Expt. Sta., St. Paul. Minn. Farm & Home Science, **12**, 1: 5. 1954.

Some good advice is offered to the prospective farmer who must establish his first farm. R. W. Hunt

257. Productive life-span of dairy cattle. R. B. BECKER, P. T. ARNOLD, and A. H. SPURLOCK, Fla. Agr. Expt. Sta., Gainesville. Bull. 540. 1954.

The largest single source of loss among 2,182 cows was udder injuries and complications. Low production and reproductive troubles were second and third major causes. Deaths eliminated 14.2% of all cows studied. Prevention of inbreeding accounted for 34.5% of the discarded fertile bulls, while bad dispositions and low production of daughters accounted for 14.1 and 10.4% respectively. The natural services of 5,177 desirable bulls were terminated by non-infectious and infectious causes. Over 25% became sterile, while advancing age accounted for only 8.8% of those discarded. R. W. Hunt

258. Ohio farm leases. J. I. FALCONER and H. R. MOORE, Ohio Agr. Expt. Sta., Wooster. Research Bull. 749. 1954.

This publication brings attention to what should be included in a good lease. Some of the major provisions are (1) provide for and allow profitable farming; (2) give a fair division of returns between land owner and operation; (3) provide for a system of farming which will maintain or increase the productivity of the farm; (4) give as much assurance as possible to the tenant that, if a good job of farming is done, the lease will continue over a period of years; and (5) be in writing. R. W. Hunt

259. What is it worth? Forage crop irrigation. R. B. ALDERFER, N. J. Agr. Expt. Sta., New Brunswick. N. J. Agr., **36**, 6. 1954.

In most cases, forage crop irrigation is not profitable in New Jersey. R. W. Hunt

260. Valve device for milker claws. E. L. TORGERSON (assignor to J. J. Hyde). U. S. Patent 2,702,526. 3 claims. Feb. 22, 1954. Offic. Gaz. U. S. Pat. Office, **691**, 4: 496. 1955.

A design for a valve in combination with a milking machine claw is described. R. Whitaker

261. Milk cooler. W. F. BAKKE (assignor to Sub-Zero Freezer Co.). U. S. Patent 2,700,281. 2 claims. Jan. 25, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 4: 499. 1955.

A combination refrigerated dry storage cabinet and open top cooler for milk cans. A refrigeration unit with submerged coils cools water which is rapidly circulated from a chilled

water storage tank through a distributing pipe which allows the chilled water to cascade down the outside of the neck and side walls of the cans. R. Whitaker

262. Cow feeder. J. W. Shipp. U. S. Patent 2,701,667. 3 claims. Feb. 8, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 2: 253. 1955.

A container for holding dry cow feed, open on top and with a hinged bottom. The bottom or gate may be kept open or closed, depending on the position of a rod extending through the container and pivotally attached to the gate. R. Whitaker

263. Bovine twitch. L. J. Rafoth. U. S. Patent 2,701,550. 2 claims. Feb. 8, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 2: 224. 1955.

A device for restraining cattle. R. Whitaker

264. Vacuum container milking system and apparatus therefor. G. R. Duncan (assignor to Zero Manufacturing Co.). U. S. Patent 2,702,019. 16 claims. Feb. 15, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 3: 354. 1955.

A reservoir for milk coming from milking machines. The product is cooled and maintained under vacuum. R. Whitaker

ICE CREAM

265. Low-lactose concentrate makes better ice cream. J. J. Sampey, Abbotts Dairies Inc., and C. E. Neubeck, Rohm and Haas Co., Philadelphia. Food Eng., **27**, 1: 68. 1955.

Hydrolysis of lactose in concentrated milk products by Lactase B, an enzyme preparation from yeast, offers possibilities for product improvement. The enzyme usually is used at the rate of 1% of the lactose content. The rate of hydrolysis depends on holding temperature and will occur at some storage temperatures. At 35-38° F. about 5% of the lactose is hydrolyzed in 7-10 d. At 111° F. about 15% is hydrolyzed in 2 hr. No special equipment is necessary. In pilot plant trials ice cream mix was made with 13-15% MSNF from concentrated skimmilk in which 20-30% of the lactose had been hydrolyzed. Fat content was 10-12%. About 1% less sucrose than usual was used. In the ice cream, texture and flavor were improved and no sandiness was evident after 4 mo. cabinet storage. Control lots with equivalent MSNF showed sandiness in 2-4 wk. T. J. Claydon

266. Distribution of ice cream on rural routes. E. Thom. Ice Cream Rev., **38**, 7: 48. 1955.

Several ice cream companies located in Ill., Wis., and Iowa are selling ice cream and other dairy products directly to rural families. Experience gained with the rural delivery of ice cream indicates that the operation is best adapted to the smaller company with a limited number of dealers and located in a predominantly rural area. W. J. Caulfield

267. Latest analysis of counter freezer and soft-serve gallonage. Ice Cream Trade J., **51**, 2: 15. 1955.

Latest reports indicate that 20,000 counter freezers and soft ice cream outlets in 1953 made and sold on the premises 80,865,000 gal. of frozen products. The volume was 10.7% of the total 1953 production of ice cream and related products. Hard and soft ice milk accounted for 28,446,000 gal., 35.2% of the total 80-million gal. Leading states in ice milk production were Calif., Ill., Iowa, Kan., Nebr., Ohio, Tenn., and Wash. W. H. Martin

268. Location is the most important single factor. J. J. Sheuring, Univ. of Ga., Athens. Ice Cream Trade J., **51**, 2: 42. 1955.

Location of the store is the most important single factor in the success of the soft-serve type ice cream or ice milk store. The store should be located in an area where there are plenty of potential customers; usually a location in the middle-class area is desirable. The lot should be on a well-traveled local street and have rather slow traffic. Super-speed highways, especially those with divided lanes, should be avoided, unless the store is at some intersection where all traffic must stop. A corner location at a stop-light intersection of a main highway and a busy local street is ideal if the neighborhood around it is reasonably built-up. The lot should be available from all directions of traffic flow. The lot should be level, well-drained, shaded if possible, surfaced and have a minimum of 10,000 square feet of area if motorists are expected as customers. W. H. Martin

269. Trends in ice cream costs. Ice Cream Trade J., **51**, 1: 18. 1955.

The weighted average cost per gal. of ice cream for 1953 was 155.15¢, compared with 159.50 in 1952, a decrease of 4.33¢ per gal. Decreases occurred in ingredients costs down 8.09¢, administrative expense down 8.59¢, selling and advertising costs up .71¢, delivery costs up 1.55¢, and customer service was up .9¢ per gal. Lower costs were attributed to lower product costs, resulting from a transition of volume from ice cream to lower butterfat products. Since 1944, the total cost per gal. of ice cream has steadily increased from 86.28¢ per gal. to the 1952 high of 159.50¢. W. H. Martin

270. Sherbet up 2% in 1954. Ice Cream Trade J., **51**, 2: 18. 1955.

The volume of sherbet produced in the U. S. in 1954 was 31,420,000 gal., up 2% from the 1953 volume. Production in June, July, and August amounts to 40% of the year's total; however, the volume for the remaining months indicated that sherbet is having more of a year-round acceptance. W. H. Martin

271. Soft serve—A look at this new and fast-growing industry. J. J. Sheuring, Univ. of Ga., Athens. Ice Cream Trade J., **51**, 1: 26. 1955.

Composition, nutrient content, and food value of soft-serve frozen desserts are discussed.
W. H. Martin

272. Tray and stick holder for frozen confections. E. W. Larkin. U. S. Patent 2,702,-011. 1 claim. Feb. 15, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 3: 352. 1955.

Sticks are held in position over mold pockets by a strap which runs lengthwise on a multiple pocket mold for frozen confections. The sticks are held in place as the holes are very slightly larger than the stick and the sides of the holes are roughened. R. Whitaker

273. The purchase of mix against making your own. L. R. Dowd, Univ. of Connecticut. Ice Cream Trade J., **51**, 1: 40. 1955.

Factors to be considered in buying or making ice cream mix are presented. A volume of 10 cans per week or 1,000 cans per year is suggested as the minimum amount for a profitable mix making operation. If the margin between the cost of mix and making it is in excess of $3 per can, it may pay to make mix; if the spread is $2 or less, it is probably more profitable to buy mix. W. H. Martin

PHYSIOLOGY AND ENDOCRINOLOGY

274. Reviews of the Progress of Dairy Science: Section E. Diseases of Dairy Cattle. P. S. Blackburn. **Section A. Physiology of Dairy Cattle.** E. C. Owen. J. Dairy Research, **21**, 3: 391. 1954.

The diseases specifically discussed in Section E are mastitis and contagious abortion. Under mastitis are discussed treatment, diagnosis, experimental mastitis, mastitis caused by organisms other than *Streptococcus agalactiae* and a general section. The bacteriology, diagnosis, vaccination, and a general section are included in the section on brucellosis, and 150 papers of recent origin are covered. In Section A, the physiology and biochemistry of rumination is covered in sections specifically devoted to various phases of this topic. This quite comprehensive report includes 560 articles, mostly from abroad. J. D. Donker

275. Physio-chemical state of circulating steroids. F. Bischoff, R. D. Stauffer, and C. L. Gray, Santa Barbara Cottage Hospital, Calif. Am. J. Physiol., **177**: 65. 1954.

The distribution coefficients for testosterone, estrone, estradiol, and progesterone between 6% bovine albumin solutions and peanut oil indicated that albumin plays a greater role than fat in the transfer of free steroids in blood serum. No evidence for conjugated steroid proteins was found. Human blood serum taken during the last trimester of pregnancy contained 2.5 μ estradiol equivalents per 100 cc. and it was in the free state. R. L. Hays

276. Changes in uptake of radioactive iodine by the thyroid of the rat, during the estrous cycle. F. A. Soliman, Cairo Univ., Egypt, and E. P. Reineke, Mich. State Coll., East Lansing. Am. J. Physiol., **178**: 89. 1954.

The radioactive iodine content of the thyroids was determined 6 hours after the intraperitoneal injection of 1 μc. of I^{131}. The uptake was significantly increased during estrus. The food intake of iodine was controlled. Injection of small doses of estrogen increase the iodine uptake by the thyroid, and progesterone depresses it. It is suggested that the rhythmic changes in thyroid function during the estrous cycle are caused by the levels and proportions of estrogen and progesterone secreted by the ovary. R. L. Hays

277. Effects of age upon the physiological behavior of calcium in cattle. S. L. Hansard and C. L. Comar, Univ. of Tenn., Oak Ridge, and G. K. Davis, Fla. Agr. Expt. Sta., Gainsville. Am. Physiol., **177**: 383. 1954.

The absorption, distribution, and elimination of oral or intravenously injected labeled calcium in 36 Hereford cattle varying in ages from 10 days to 190 months were studied. The elimination of unabsorbed calcium in the feces was 3%, 6%, and 62% for the 10 day, 1 month, and 6 month old animals, respectively. The amount increased to 83% in the 12-15 year old group. The rate of exchange of calcium ions between the blood and the rest of the body decreased significantly with age. R. L. Hays

278. Immediate blood cell count response to epinephrine in splenectomized dogs. L. H. Hamilton and S. M. Horvath, Univ. of Iowa, Iowa City. Am. J. Physiol., **178**: 58. 1954.

By comparing the blood cell counts of samples taken from the aorta, caudal vena cava, and the right ventricle of normal and splenectomized dogs before and after the intravenous injection of 0.5 mg. of epinephrine, it was found that there was no erythrocytosis in splenectomized dogs, but there was in normal dogs. Up to 2 min. after epinephrine injection, there was no difference in leucocyte counts in the two groups of animals, but splenectomy may have decreased the response to epinephrine during the period 2-5 min. after injection. The evidence indicates that at least part of the increase in leucocytes and erythrocytes due to epinephrine injection comes from the pulmonary circulation. R. L. Hays

279. The mammary gland spreading factor. J. R. Elliott and C. W. Turner, Mo. Agr. Expt. Sta., Columbia. Research Bull. 537. 1953.

As a means of investigating the role of hor-

mones in stimulating the growth of mammary glands, albino rats, albino mice, guinea pigs, and rabbits were used as experimental animals. The amount of spreading or growth factor present in the glands was found to increase until a peak of 1/2 to 2/3 of the gestation period was reached. The injection of estrogen, and progestrone, pituitary extracts, particularly a fraction rich in lactogenic hormone, testosterone, desoxycorticosterone acetate, cortisone acetate and relaxin were all shown to be effective in causing elaboration or activation of the mammary gland spreading factor. The theory is presented that the amount of spreading factor is related to the number of epithelial cells in the mammary gland. R. W. Hunt

280. The secretion of a single tracer dose of labelled iodide in the milk of the lactating cow. R. F. Glascock, Natl. Inst. Research Dairying, Univ. Reading., J. Dairy Research, **21**, 3: 318. 1954.

Upon administration of a single dose of radioactive potassium iodide (I^{131}), milk collections were made and analyzed for activity. The most activity was found the first day and by the 15th day had decreased to about 0.5% of the maximum value. At this time, less than 5.0% of the activity had been found in the milk. Urine contained much higher activities. The activity was found in the iodide form in the milk. J. D. Donker

281. Electrical activity of single muscle cells of pregnant uteri studied with intracellular ultramicroelectrodes. J. W. Woodbury and D. M. McIntyre, Univ. of Wash., Seattle. Am. J. Physiol., **177**: 355. 1954.

The membrane potentials of cells of uteri from pregnant humans, rabbits, cats, and guinea pigs were low as compared to striated, cardiac, and other smooth muscle. Oxytocin caused a reversal of the steady membrane potential in some but not all of the cells. The evidence indicates that the lowered membrane potentials during pregnancy are probably due to an increase in the permeability of the membrane to sodium and the reversal caused by oxytocin is due to a marked increase in the permeability to sodium. R. L. Hays

SANITATION AND CLEANSING

282. Method and apparatus for cleansing milking machines. E. Domingo. U. S. Patent 2,702,767. 10 claims. Feb. 22, 1955. Offic. Gaz. U. S. Pat. Office, **691**, 4: 558. 1955.

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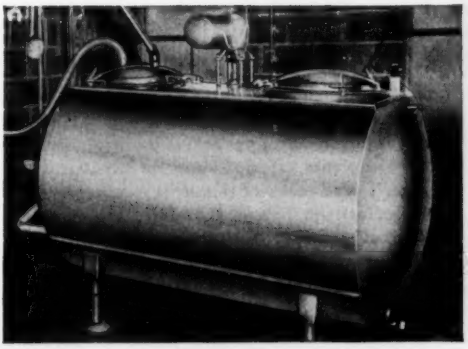

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